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THE STRUCTURE AND DEVELOPMENT OF THE NEPHRIDIA OF ARENICOLA CRISTATA STIMPSON

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF ZOÖLOGY)

BY

RALPH S. LILLIE

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The Structure and Development of the Nephridia of Arenicola cristata Stimpson.

By

Ralph S. Lillie.

With Plates 22—25 and one Textfigure.

1. Introductory.

The nephridia of *Arenicola* were first suggested to me as a subject of anatomical and embryological investigation by Dr. C. O. Whitman, during the season of 1896 at the Marine Biological Laboratory of Wood's Hole. The work then begun was subsequently interrupted for a time; and the greater part of the work described in the following paper was completed during the years 1898—1901, at the Marine Biological Laboratory and in the Hull Zoölogical Laboratory of the University of Chicago.

I take pleasure in expressing my best thanks to Dr. Whitman for his continued interest and criticism. My thanks are also due to Dr. C. M. Child of the University of Chicago, for much helpful information, especially with reference to the early development; and to my brother, Professor F. R. Lillie of the same University for constant and valuable assistance.

The nephridia of several species of the genus *Arenicola* have been at various times figured and briefly described in systematic or anatomical treatises; and in several instances have been made the subject of special investigation. Claparède (1868) and Fauvel (1899) have given brief descriptions of the nephridia of *A. Grubii* and *A. ecaudata* respectively. The greater number of such studies have been made on *A. marina*; investigations on the nephridia of this form have been made by Cosmovici (1880), Cunningham (1887), Benham (1891, 1893), Kyle (1896); and more recently by Gamble &

Ashworth (1898, 1900), who in their two important papers on the anatomy and affinities of the Arenicolidae have added materially to our knowledge of these organs. The second paper of these authors (1900) contains a comparative account of the anatomy, relations, and vascular supply of the nephridia of five species of the genus, viz: — *A. marina* Linn., *A. cristata* Stimpson, *A. Claparedii* Levinsen, *A. Grubii* Claparède and *A. ecaudata* Johnston; the authors have also given a summary of our present knowledge of the anatomy and histology of the adult nephridia, and have added a brief account of the condition of the organs in earlier developmental stages, so far as this has as yet been ascertained.

Our knowledge of the larval development of *Arenicola* has, however, until recently been too limited to admit of a detailed study of the mode of origin of the nephridia, and of the condition of these organs at different periods in the life-history. Benham (1893) and Gamble & Ashworth (1898, 1900) have contributed a few observations on the nephridia of certain post-larval stages. Their observations were made on relatively few specimens which in all cases were well-advanced in development. Accordingly, we have had as yet no detailed account of the origin, growth, and histological differentiation of the nephridia in *Arenicola*. One reason for this deficiency seems hitherto to have been the difficulty of obtaining the material for such an investigation. In my own studies, however, instead of depending upon the slow and precarious methods of digging and tow-net collecting, I have found it more practicable to rear the larvae artificially; in this manner it has been found possible to secure all the material necessary for a detailed examination of the larval development from the early free-swimming period up to a stage at which the adult characteristics are essentially complete.

The aim of the present study has been to complete so far as possible our knowledge of the origin and histological differentiation of the nephridia, and to determine to what extent the peculiarities of the adult nephridium gain an explanation through the conditions of larval development. The relations of the nephridia to the body-segmentation and to the septa, and the manner of development of the different portions of the organ — nephrostome, glandular portion and terminal vesicle (whether separately, or by differentiation from a single embryonic rudiment) — are matters that have received especial attention. There has also been added a brief account of the anatomy and histology of the adult nephridium, draw-

ing especial attention to a few peculiarities that have hitherto apparently escaped observation.

2. Technique.

The adult nephridia are best prepared for histological study by fixing in HERMANN'S fluid for 15 to 30 minutes, and then transferring immediately to MERKEL'S fluid (WHITMAN'S modification, with equal parts 1% chromic acid and 0.25% platinic chloride), where they are allowed to remain for one to three hours. They are then washed in distilled water and transferred to alcohol as usual. The treatment with MERKEL'S fluid prevents excessive blackening; the fixation is apparently very faithful and the cilia are well preserved. The sections were mostly 7.5 μ in thickness. The reduced osmium may be still further removed from the sections by leaving the slides over night in a mixture of alcohol and hydrogen peroxide (usually composed of about one part commercial H_2O_2 to three parts strong alcohol, mixed immediately before using). The tissues are by this treatment almost entirely freed of the osmium and left in a suitable condition for staining. The most satisfactory stain was found to be HEIDENHAIN'S iron haematoxylin, with a counter-stain of erythrosin. For study with oil-immersion objectives, the best mounting medium proved to be thickened cedar-oil (immersion oil). This medium hardens slowly, but has the advantage of producing a perfectly homogeneous and transparent lacquer of great permanence and of almost the same refractive index as that of the oil in which the lens is immersed. Very clear and sharp images are thus obtained with the highest powers.

The larvae were fixed by a great variety of methods, of which only a few proved satisfactory. Treatment with 1% osmic acid for 2 to 5 minutes, followed by MERKEL'S fluid for 1—3 hours, gave fair preparations in many instances. The best fixative for general purposes proved to be HERMANN'S fluid (2—5 minutes), followed by MERKEL'S fluid (1—3 hours). In most instances, therefore, the larvae were fixed by the HERMANN-MERKEL method, and the sections (mostly 5 μ in thickness) were stained chiefly with iron haematoxylin and erythrosin as above described, and mounted in cedar-oil. Very clear and sharply differentiated preparations were thus obtained. The tissues were remarkably well preserved, delicate structures

such as cilia retaining, in favorable preparations, almost the normal appearance.

Young larvae of the swarming stage were best prepared by fixation in HERMANN'S fluid for two minutes, followed by MERKEL'S fluid for one hour. It is necessary to imbed such larvae as soon as possible after fixation; otherwise the yolk becomes so brittle as to render it practically impossible to secure unbroken sections. If imbedded and cut immediately after fixation, no such difficulty is experienced.

The succeeding descriptions are based upon the examination of a very large number of preparations, and all possibility of deception arising from imperfect preparations, abnormalities of structure in individual larvae, or failure to observe critical stages, has, it is hoped, been avoided.

3. General account of the larval Development with Especial Reference to Segmental Structures.

Arenicola cristata, Stimpson, the species upon which the entire following study has been made, is found in several localities in the neighborhood of Wood's Hole — most abundantly at North Falmouth, where the extensive sand-flats furnish ideal conditions for its development. The animals are found burrowing at a depth of 6 to 18 inches in the sand, which is deeply saturated with decaying organic matter; and in the summer months are in nearly all instances well developed and of large size (6—12 inches in length). For the larval stages, it has proved necessary to rely upon material reared in the laboratory, for it has been found impossible in any other way to obtain the requisite quantity of young larvae. I have frequently searched the sand with a lens in the hope of finding young Arenicolae, but always without success; the larvae, however, lend themselves so readily to artificial rearing that no difficulty has been experienced in securing in this manner an abundance of specimens of all stages.

The general characteristics of the adult *A. cristata* are sufficiently known from the descriptions of STIMPSON, VERRILL, GAMBLE & ASHWORTH, and others. The young free-swimming larvae have been briefly described by WILSON (Johns Hopkins Studies, 1883) and CHILD (Z. Bull. Vol. 1, 1897). In the present paper I shall give merely a short account of the general characteristics of the larval stages, omitting details that do not immediately bear on the problems under

consideration. Child (1900) has given an accurate account of the early development within the egg-membrane. My own descriptions will have reference to the metamorphosis and larval development up to a period at which most of the definitive external characteristics are complete.

Very little care is needed in order to preserve the larvae for weeks or even months in a healthy condition, and capable of growth and development. The egg-strings are placed in clean sea-water in large, flat, well-lighted dishes covered by sheets of glass to prevent excess of evaporation, and containing a few pieces of *Ulva* for aëration. It is advisable to change the sea-water at intervals of a week or ten days; otherwise the dishes require very little attention. The organic debris present, derived from minute Algae, particles of decaying *Ulva*, and the bodies of dead larvae, seem to furnish the larvae with sufficient food for the development of a fairly large proportion of their number. Under these conditions, larvae have been kept in the laboratory for periods of 14 to 15 weeks, apparently in a perfectly healthy condition and exhibiting all the normal activities. Development seems, however, to progress more slowly than under the natural conditions, for the largest specimens reared never exceeded a length of 15 mm., although exhibiting in other respects an almost perfect agreement with the adult in appearance, anatomical structure, and behavior. The food-supply is in all probability insufficient for rapid growth; this is indicated by the fact that if kept in sea-water to which carmine powder has been added the larvae usually exhibit a greatly increased rate of growth, especially in the early stages. It would no doubt be possible, by the employment of suitable methods of feeding, to rear them to more advanced stages than the above.

The larvae leave the egg-strings in from two to three days after oviposition, in the form of slightly elongated maggot-like free-swimming organisms (about 0.3 mm. in length), which exhibit a most pronounced positive phototaxis combined with negative geotaxis. As a result of these tendencies they swim rapidly to the light side of the dish and there gather in enormous numbers at the surface of the water. At this stage a larva possesses in addition to the peristomium (which is without setae), three setigerous trunk somites, in each of which are two paired sets of setae corresponding to the notopodial and neuropodial setae of the adult. The notopodial setae of each side are generally two in number, spoon-shaped and spear-

shaped respectively, and are elongated and already capable of a very considerable degree of protrusion and retraction. The neuropodial setae consist of a single short hook on each side of the ventral surface of each setigerous somite. As growth proceeds, additional hooked setae appear ventrally to those already laid down; at the swarming stage, however, a single hook only is as a rule present on either side in each somite. In the third setigerous somite, the setae in the majority of larvae are as yet incompletely formed; as elongation proceeds, however, they enlarge and acquire the same characteristics as those of the preceding somite. Locomotion in the swarming stage is effected by means of the two ciliary rings, prototroch and paratroch; these are connected by a median ventral band of shorter and stouter cilia. The continued activity of the cilia propels the larvae at a uniform rate in a forward direction toward the source of light. On the prostomium are two simple "eye-spots", each of which consists of a compact clump of pigment or excretory granules on the surface of the brain. Large clear cells, apparently of a glandular nature, occupy a large portion of the prostomium, and similar but smaller cells are found at the posterior extremity of the body. These cells probably furnish, in part at least, the glutinous material by the aid of which the larvae later form the rough tubes in which they live subsequently to the termination of the swarming period. In fixed and stained preparations, the cells have a clear and reticulate appearance (Figs. 7 and 8, Plate 22); they diminish and disappear shortly after the beginning of the crawling and burrowing period.

The swarming stage apparently serves simply for the dissemination of the individuals of the new generation, and differentiation of internal organs remains very incomplete until after its termination. The intestine at this period still consists largely of yolk, and its lumen is as yet very incompletely formed. There is, however, a partial subdivision into oesophagus, stomach and rectum — a subdivision which becomes well defined shortly after the beginning of the crawling stage, and persists through part of the larval period and into adult life. Mouth and anus have not yet appeared; the anterior part of the intestine, however, which later forms an eversible proboscis, exhibits already a relatively high degree of differentiation (Fig. 7) — a peculiarity in evident correspondence with the early appearance of its activity and the important part which it plays in larval life. The brain, oesophageal ring and ventral

nerve cord, are continuous with one another and are well defined in the ectoderm of the anterior region of the body. Posteriorly, the ventral cord is less distinctly delimited from the rest of the ectoderm, passing gradually into the undifferentiated tissue of the growing zone. Numerous longitudinal muscle-fibres are closely applied to the inner surface of the body-wall, and fibres derived from this layer are already attached to the inner ends of the seta-sacs. No circular fibres, however, have as yet appeared; these arise later in development and apparently in an entirely different manner from the longitudinal fibrils, i. e. they appear in the ectoderm while the longitudinal fibres are somatopleuric (see below).

The free-swimming stage lasts for a period whose exact duration, in the case of an individual larva, is difficult of determination, but is probably from one to two days at normal summer temperature. At its close the larvae undergo the change which has been described as a metamorphosis: they lose their cilia, sink to the bottom, and adopt a crawling and burrowing and partially tubiculous mode of life which lasts for the remainder of their existence. This change takes place at about the time of formation of the fourth setigerous somite. The larva represented in longitudinal section in figure 8 (Plate 22), is undergoing the transformation: it has already lost most of its cilia; the mouth opening has broken through, and the proctodaeum is in process of formation: the intestinal yolk is evidently undergoing absorption, and the lumen has become more distinct.

The intestinal lumen quickly becomes spacious and continuous from end to end of the body; and in larvae of 6 or 7 somites the yolk has largely disappeared, and the intestine has begun its functional activity. The proboscis, by its continual eversion and retraction, fills the intestine with the surrounding débris, playing at the same time an important part as a burrowing organ, in which form of activity it is assisted by the action of the setae and the muscles of the body-wall. From now on, the habits remain uniform and seem in all essential respects identical with those of the adult *Arenicola*. The material introduced by the proboscis into the intestine is passed through the latter, and the contained organic matter furnishes the sole food-supply of the developing larvae.

With the assumption of the definitive life-habits is associated a change in the reactions towards light and towards the contact of solid bodies. The larvae become negatively heliotropic, exhibiting

a marked tendency to become oriented with the anterior end directed away from the source of illumination, and thus eventually, as a result of the continued crawling movements, to gather in regions remote from the source of light. The other marked peculiarity which now first makes its appearance is a tendency to bring as large a portion as possible of the body-surface into contact with solid bodies (positive stereotropism or thigmotaxis). Larvae freed from adhering solid particles, and placed in a vessel containing loose finely divided débris of any kind, invariably exhibit restless squirming and crawling movements, which continue until the body is once more in contact on all sides with solid particles. When these particles consist of loose sand-grains, diatom-shells or similar light, finely-divided material, the result is that the animal soon becomes enclosed in a rough tube formed by the adhesion of such particles to one another and to the body-surface by means of the abundant secretion furnished by the ectodermal glands. In this manner are formed the rough tubes in which the remainder of the larval period is passed. When the larva is freed from such a tube, the restless squirming movements are resumed, and continue until another tube has been formed. Tendencies such as these, when combined with the characteristic negative heliotropism, will, under natural conditions, plainly favor burrowing movements of the kind observed. Both forms of reaction apparently persist until adult life, and through them the characteristic behavior of the animal is largely determined.

After the close of the swarming period and the assumption of the burrowing habit of life, growth and elongation proceed steadily and uniformly until the full number of somites have been formed. Elongation is due, as in other Annelida, to the activity of a posterior growing zone, which occupies a position immediately anterior to the original situationof the paratroch (Figs. 7—10, Plates 22 and 23; 29—33, Plates 23 and 24). The large clear mesodermal and ectodermal cells of this region contain large nucleolated nuclei, frequently found in process of mitosis, and continually give rise anteriorly to new cells which constitute the basis of the newly forming somites. As each somite appears, it is divided off from the one next succeeding by a mesodermal septum; the growing zone thus retains unaltered its distinctive characters, and occupies a constant position relatively to the posterior end.

The short region posterior to the growing zone post-mesodermal

region or pygidium) retains its characteristic peculiarities in an almost unaltered form throughout the whole of the development (Figs. 7—10, Plates 22 and 23; 29—33, Plates 23 and 24). Its ectodermal cells are small and vacuolated, and contain a granular yellow or brownish pigment, evidently of an excretory nature, which seems to accumulate in this region as the animal grows older — possibly because of its isolation from the rest of the larval body, and its lack of vascular supply. A sharp line of demarcation exists between the ectoderm of this region and that of the growing zone immediately anterior to it. The portion of the intestine contained in this region corresponds to the proctodaeum, and is separated by a valve from the entodermal portion immediately in front (Fig. 33, Plate 24). In its ventral wall is inserted a bunch of strong stiff cilia.

While the posterior portion of the larval body is undergoing elongation in this manner, differentiation of the more anterior somites is in progress. In young larvae with fewer than nineteen somites, each somite possesses at its earliest appearance — in addition to its section of the intestine and the newly formed muscle-fibres of the body-wall — the rudiments of dorsal and ventral setae; and is separated from its neighbors by complete mesoblastic septal partitions (Figs. 9, 10; 29, 30, 31, 32). As growth proceeds, the septa become incomplete dorsally, and the adjacent body-cavities become continuous with one another. At the same time the setae become fully formed and functional; and the supra- and sub-intestinal blood-vessels, originally simple spaces between the opposed and thinned-out walls of the early mesoblastic somites (Figs. 8, 9), become well defined and of uniform diameter throughout.

The stomach, as described above, is at an early stage (see Figs. 7, 8, Plate 22) already sharply distinct from the oesophagus. Throughout the period now under consideration from 8 to 18 somites), the oesophagus undergoes a continual backward prolongation in a manner presently to be described, and its region of junction with the stomach is thus gradually shifted backward from the second (Fig. 8) to the seventh somite, its definitive position. In histological structure, the walls of the oesophagus consist chiefly of a single layer of densely ciliated cubical epithelial cells. The stomach is of greater diameter than the oesophagus, and its walls, which are much thicker ventrally than dorsally, are composed of very characteristic large uniformly stained cells of homogeneous appearance. Along its ventral wall extends a ciliated groove continuous anteriorly with

the ciliated cells of the oesophagus and posteriorly with those of the rectum. Posteriorly, the stomach merges gradually in earlier stages into the undifferentiated posterior portion of the intestine; later its posterior limit becomes defined, though never with absolute precision: this limit is in the tenth or eleventh somite, where the stomach passes without abrupt transition into the posterior narrower portion of the intestine. This latter region (rectum) is in its anterior portion completely lined by ciliated cells continuous with those of the stomach; further backward the cilia become restricted to a longitudinal groove along the ventral wall; and as the growing region is neared, the cilia finally disappear, and the wall becomes composed of large clear cells few in number in cross section, and containing large nuclei (Figs. 21, 24, 30, Plate 23).

Both stomach and oesophagus are suspended to the dorsal body-wall by a mesentery in which runs the supra-intestinal blood-vessel. The sub-intestinal vessel runs along the ventral surface of the intestine, and presents a characteristic appearance from the large granular chloragogen cells which invest it. Posteriorly these cells merge gradually into the undifferentiated mesoderm cells of the growing zone (see Figs. 9, 10, 16, 17 etc., 30, Plates 22 and 23).

For the greater part of the above period the septa form incomplete transverse partitions extending obliquely backward from the ventro-lateral regions of the corresponding somites to the sub-intestinal blood-vessel. Each septum from the third to the tenth inclusive differs from the rest in being associated with the development of a pair of nephridia, which arise on either side in the mesoblast at the time of formation of the septum, and in continuity with the posterior face of the latter. Each nephridium which thus arises soon acquires the form of a minute intracellular tubule in the anterior region of the somite, opening through the septum into the body-cavity beyond by an aperture through which cilia project. The two earliest formed pronephridia, those of somites IV and V, persist for a short time only, and degenerate at a comparatively early period in the development. The remaining six pairs (in somites VI to XI inclusive) are directly transformed into the definitive adult nephridia. The somites behind the eleventh never give rise to such pronephridia, so far as my observation has extended. It is possible that pronephridia may occasionally appear in these somites, as might be expected from the fact that certain species exist (e. g. *A. caudata*) in which nephridia are normally present

in these somites: but of the numerous larvae that I have examined, not one has presented this condition, so that its occurrence is at any rate exceptional. The pronephridia of somites IV and V, on the contrary, appear with perfect constancy as normal features of the early development.

In other respects the development of the post-nephridial somites takes place (until the nineteenth somite is reached) in exactly the same manner as that of the more anterior somites as already described. The result is the formation of a chaetigerous anterior body-region which includes the first eighteen somites. The primitive septa are, as above explained, dorsally incomplete, so that the body-cavities communicate freely with one another throughout the greater part of this region. By the time of attainment of the nineteenth somite it is found, however, that the four most anterior somites have become separated from the others by muscular partitions which have arisen at the posterior boundaries of the first, third and fourth somites. These structures are the fore-runners of the three diaphragms of the adult: they do not represent the primitive septa of this region, but are formed independently of these in a manner presently to be described. The body-cavity from the posterior boundary of somite IV to the anterior boundary of somite XIX, is entirely without septa, and remains in this condition throughout life, forming the spacious undivided coelom characteristic of the Arenicolidae.

With the appearance of somite XIX, a marked change takes place in the character of the segmentation. The septum between somites XVIII and XIX remains complete, forming a membranous partition which persists throughout life and divides the anterior chaetigerous body-region from the region formed behind this limit. The somites now formed are narrower and shorter than those of the anterior region (see Plate 24, Fig. 33); they are furthermore entirely destitute of setae, and are separated from one another by complete septa. At the beginning of the time of formation of this region, its somites are not conspicuously narrower or shorter than those of the anterior region. As growth proceeds, however, the characteristic differences between the two regions become more evident, and are well marked by the time the total number of somites has reached the neighborhood of thirty. With a further increase in the number of somites, the dividing line becomes better defined, until finally, in larvae of 2—2.5 mm. length, the distinction between the two regions

is as distinct as in the adult. The posterior achaetous region undergoes relatively little further differentiation, and its somites exhibit throughout life an almost complete resemblance to one another. The addition of new somites continues in the usual manner until the definitive number has been attained, after which the growing zone loses its distinctive characters and becomes indistinguishable as such.

The disappearance of the growing zone and the cessation of somite-formation take place with great regularity at a stage of about 58 somites. The final number of achaetous somites is thus found to be approximately constant; it may be accurately determined by counting the septa in longitudinal sections of specimens in which the growing zone has disappeared. In such larvae the number is found to range from 38 to 40, and appears never to exceed or fall below this. The total number of somites formed in this species of *Arenicola* seems thus approximately constant (from 56 to 58); in adult specimens, however, the number actually found is generally conspicuously smaller than the above; the deficiency is readily accounted for as due to a loss of a portion of the posterior region by autotomy, which is of frequent occurrence in this section of the body.

Although the posterior region increases in absolute size with the growth of the animal, it undergoes little further differentiation, and remains throughout life in a primitive uniformly segmented and achaetous condition. The presence of both dorsal and ventral mesenteries, the regular repetition from end to end of complete septa, the simple uniform structure of the intestine and the regular metameric repetition of the circum-intestinal blood-vessels, are other characteristic features of this region all of them of a decidedly primitive kind. All of these features appear early in the development of the region, and persist throughout life.

With the anterior chaetigerous region the case is very different, and very extensive further alterations are necessary before the definitive structure is finally attained. Fig. 30, Plate 23 represents a sagittal section of a larva at a stage of about 8 somites. The metamerism, it will be observed, is expressed in a relatively simple manner: the ventral ectoderm is thickened at segmental intervals, and the septa, all of which are incomplete dorsally (except those just forming), extend from a region a little in front of the constriction upwards and obliquely backwards to the sub-intestinal blood-vessel. The first septum is inserted into the oesophagus immediately behind the proboscis, and into the body-wall almost at

the plane of insertion of the retractor muscles of the proboscis. The oesophagus extends backward through the second somite and joins the stomach near the anterior limit of the third somite; it is evidently undergoing elongation, as may be seen by comparing its extension at this stage with that found in the earlier stages represented in Figures 7 and 8. This backward prolongation is further indicated in the fact that the septa, which in earlier stages occupied planes approximately perpendicular to the long axis (Plates 22 and 23, Figs. 7, 8, 10), have acquired a pronounced slant upwards and backwards. It is to be carefully noted that at this stage the second septum is inserted almost at the anterior boundary of the stomach, while the third and fourth septa, whose line of insertion into the body-wall corresponds with that of the later formed diaphragms, are attached to the ventral blood-vessel at points far behind the anterior boundary of the stomach.

As the oesophagus elongates, the stomach is, as it were, pushed backwards, and the above mentioned backward slope of the septa becomes more marked (Plate 24, Fig. 36). This backward growth has the further result that when the second and third diaphragms are formed (in a manner shortly to be described), each becomes inserted into the oesophagus at a position well in advance of the stomach, although as just shown, this organ originally extends far in advance of the region finally occupied by the oesophagus. The stomach seems during these changes of position to undergo an extensive histolytic disintegration and reorganization to form the tissue of the oesophagus — a fact which must also be taken into consideration in accounting for the changes in the position of septa and diaphragms. These histolytic changes will shortly be described.

The most anterior septum occupies from the first a position immediately behind the line of insertion of the proboscidial retractor muscles (Plate 24, Fig. 35). As growth proceeds, the relations of these two originally independent structures become closer, and at the stage represented in Fig. 36 — in which twelve somites have been laid down — the two have become intimately associated with one another, although still distinguishable on close examination. This association of proboscidial muscles and first septum becomes in later stages more complete, and both eventually enter into the formation of the muscular first diaphragm. The posterior peritoneal wall of the adult diaphragm may be regarded as representing the original first septum.

The ventral portion of the second septum, which, like all the other septa behind the first, is intimately related to the subintestinal vessel, carries blood-vessels[1] (which are already laid down in the larva of Plate 24, Fig. 36) from the subintestinal vessel to the ventral body-wall of the second somite. These blood-vessels mark throughout life the original position of the second septum; this structure has thus nothing to do with the formation of the second diaphragm.

The second and third diaphragms, which in the adult form the posterior boundaries of somites 3 and 4 respectively, are formed independantly of the primitive septa, and represent entirely different structures from these — as shown by their method of formation, histological composition, and relations to internal organs. The second diaphragm begins its appearance at the time of formation of the eleventh or twelfth somite; and in most instances completes its extension across the body cavity during the time of formation of the next two somites. Plate 24, Fig. 36 represents the appearance in sagittal section of a larva of 13 somites, showing the appearance of the newly formed diaphragm under a low power. It will be observed that its line of insertion into the ventral body-wall coincides with that of the primitive septum. The latter structure persists for some time after the diaphragm is formed, and can be seen in its original position stretching diagonally upwards and backwards to the subintestinal vessel at the anterior border of the stomach. The diaphragm on the other hand, extends in a perfectly transverse direction completely across the body-cavity and is inserted into the oesophagus in a position a short distance in front of the stomach.

The formation of the diaphragm takes place through the interaction of several different and partly independent processes. At its first appearance (in a larva of 12 somites), it has the form of a purely membranous partition, inserted into the ventro-lateral region of the body-wall on a level with the primitive septum, and extending transversely to the oesophagus which it joins at a region

[1] Blood-vessels of essentially similar relations are later laid down in the post-diaphragmal septa; and, as will be shown later, the distribution of the segmental blood-vessels in the adult and especially their peculiar relations to the nephrostomata, are explained through the fact of their formation in the primitive septa, where they appear as spaces between the two lamellae Plate 22, Fig. 9; Plate 24, Figs. 32, 33). Since the nephridia are from the first in intimate connection with the septa, the position of the nephrostomial blood-vessels thus becomes readily intelligible. These relations will be more fully dealt with in the division treating of the development of the nephridia.

immediately anterior to the stomach. At this stage, the diaphragm is incomplete dorsally and its muscular fibres are as yet altogether unformed. Large cells resembling in appearance the mesenchyme cells of earlier stages, are applied to its lateral regions and in the neighborhood of its junction with the oesophagus. These cells evidently play an important part in the formation of the diaphragmal muscle fibres, since the latter make their earliest appearance in regions closely corresponding with the distribution of the cells. At a stage of 12—13 somites, the diaphragm has increased considerably in extent; and muscle-fibres, which from the first show a continuity with the longitudinal muscles of the body-wall, are being laid down in its lateral regions. The extension across the body-cavity has in most larvae become complete by the time of appearance of somite XIV; and an uninterrupted partition, partly muscular, partly membranous, is thus formed at the posterior boundary of the third somite.

At this stage, the essential characteristics of the diaphragm are briefly as follows: at its lateral insertion it is entered on either side by muscle-fibres, continuous with the longitudinal fibrils of the body-wall; these extend completely across the diaphragm, passing below the oesophagus and forming a broad band of muscular tissue extending directly across the body-cavity from side to side. Large nuclei, belonging to the cells above mentioned, are applied to the diaphragm in the region of the muscle-fibres; at the insertion into the oesophagus this organ is constricted, and the muscular fibres and accompanying cells are especially well developed. In its median dorsal and ventral regions, however, the diaphragm still remains membranous and non-muscular.

It seems probable, from the appearances presented at this and later stages, that the cells applied to the originally membranous diaphragm contribute to the formation of its muscle-fibres, i. e., act as myoblast cells, resembling in this respect, the early mesoblast cells of the growing region which give rise to the longitudinal muscles of the body-wall (see below). At the same time the direct continuity of the diaphragmal muscles with those of the body-wall seems to indicate that fibres from the latter are being directly prolonged into the diaphragm by a process of ingrowth. The diaphragmal muscles would on this view have two sources; if, however, the muscle-fibres of the body-wall also increase in number by the activity of myoblast cells (as seems probable), these two modes of origin would be fundamentally identical in nature.

The third diaphragm does not appear until a somewhat later stage — typically at the time of formation of the sixteenth or seventeenth somite. Its manner of formation is in all respects similar to that of the second diaphragm. Its ventral insertion coincides with that of the fourth primitive septum; and the latter, as in the case of the third septum, may persist in its original position for some time after the diaphragm has become complete.

At this stage (17 somites), the oesophageal insertion of the third diaphragm is immediately anterior to the stomach. The oesophagus has thus evidently undergone an elongation of about the length of one somite since the time of formation of the second diaphragm. In later stages a section of considerable length intervenes between the oesophageal insertion of the third diaphragm and the anterior boundary of the stomach. Evidently therefore there is in progress a backward prolongation of the oesophagus. The manner in which this prolongation takes place is peculiar: it takes place in part probably by a process of simple backward growth; but it seems to be chiefly due to an extensive histolytic transformation of the anterior region of the primitive stomach. The nature of this transformation may be determined in part from the examination of longitudinal sections of larvae of slightly later stages. In a larva of 23 somites, both diaphragms are completely formed; and in the fifth somite, a short distance behind the third diaphragm, the changes alluded to are apparently in active progress. The oesophagus is here almost occluded by what appears to be a strong constricting muscle of deeply staining circular fibres, situated at the point of junction of oesophagus and stomach. The anterior stomach-region seems to be undergoing a process of dissolution preparatory to its transformation into oesophageal tissue. The typical unaltered stomach cells are lightly staining, and homogeneous in appearance; they are non-ciliated — except in a groove along the median ventral wall (Plate 23, Figs. 16, 17, 30) — and contain a few deeply staining granules. As the region of constriction is neared, however, their appearance undergoes a marked alteration; they become greatly narrowed and elongated, and drawn out at their inner ends apparently into long tapering flagella. Still farther forward the cells are vacuolated and largely broken down. On passing still farther forward the vacuolation becomes less marked; and finally the vacuolated cells pass by a gradual transition into the ordinary cubical ciliated cells of the oesophagus.

Everything thus points to the conclusion that a disintegration and subsequent reorganization of the anterior stomach cells are in progress. In earlier stages, the region of transformation is found farther and farther forwards, and always at the junction of oesophagus and stomach. It thus moves gradually backwards as development proceeds, and the process of transformation apparently ceases only when the definitive limits of the two regions have become finally established. In later larval stages, and in the adult, the oesophagus joins the stomach near the line of separation of the sixth and seventh somites, immediately behind the oesophageal pouches. These latter organs therefore do not arise until oesophagus and stomach have attained their definitive limits.

Some space has been devoted to a description of the above histolytic changes in order to make clear the manner in which the final relations of the diaphragms become established. At the body-wall, the line of insertion of each diaphragm corresponds to that of the original septum to which it corresponds (1st, 3rd and 4th). At the intestinal insertion however, no such correspondence can exist. The whole anterior region of the alimentary canal has undergone a complete alteration from its earlier condition; and the sections of oesophagus bounded by successive diaphragms can therefore in no way correspond to the original segmental divisions. In *Arenicola* the alimentary canal fails indeed to exhibit metamerism in its anterior portions, becoming differentiated as a whole, without reference to segmental limits. In the body-wall, on the other hand, the metamerism — as indicated by the position of the setae, external body-rings, branchiae and nephropores — corresponds closely with that originally laid down in early development. These facts have an important bearing on the question of the possibility of rigorously marking off segmental limits in the adult stages of metameric animals. This can be done only in the case of certain structures in *Arenicola*: the anterior part of the intestine shows no indications of metamerism.

It will be sufficient for the purpose of this paper to give merely a brief outline of the remainder of the larval development, since its details are of interest in the present paper only in so far as they concern the characteristics of the nephridia which will shortly be dealt with at length. As growth proceeds, the division between chaetigerous and achaetous body-sections becomes well-defined, and with the completion of the diaphragms and the definite establishment

of the limits of the intestinal regions, the main characteristics of the adult body are complete. The activity of the growing zone ceases when the larva has attained a length of from 2 to 2.5 mm., at which stage the outward appearance and internal organization are essentially identical with those of the adult — further development consisting chiefly in an increase in size and in the completion of histological differentiation. In such larvae, the distribution of the blood-vessels is substantially as in the adult, and the two "hearts" connecting the gastric and subintestinal vessels at the anterior extremity of the stomach, are already differentiated and rhythmically contractile. The gills have appeared in the 7th to 17th setigerous somites (VIII—XVIII) as thin-walled evaginations of the dorso-lateral body-wall containing looped blood-vessels. The otocysts are present at the sides of the prostomium. The six nephridia are visible through the transparent body-wall as sac-like structures in the 5th to the 10th setigerous somites (VI—XI). The dorsal setae and the ventral rows of hooks have the usual characters, but have increased greatly in number as compared with earlier stages. Larvae of 10—15 mm. length have undergone little further alteration, except in respect to size and degree of histological differentiation. The gills have become more branched, the "hearts" more conspicuous and better defined, the number of setae has increased, and the external body-rings have appeared. At this stage in fact, the adult organization is essentially complete.

4. Anatomy and histology of the adult nephridium.

The six pairs of nephridia of the adult *Arenicola cristata* are situated in the 5th to the 10th setigerous somites inclusive. These somites correspond however, as seen from a comparison of early larval stages, to body-somites VI to XI, the first somite (peristomium) being without setae, and in the adult, not sharply demarcated externally from the second. Internally, however, its posterior limit is marked by the first diaphragm whose position corresponds closely with that of the original first septum. Between the first and second diaphragms intervenes a region composed of two somites; these are in early stages perfectly distinct from one another; in adult life, however, the bisegmental composition of this region is less evident; it is however, clearly indicated by the presence of two segmental tufts of notopodial setae, as well as by certain peculiarities of the

vascular system see Gamble & Ashworth, 1900, Fig. 26). The second diaphragm thus constitutes in reality the posterior boundary of somite III, while the third diaphragm similarly delimits somite IV. This disposition of the diaphragms is characteristic of all the species of *Arenicola* that have thus far been examined, and has its origin in *A. cristata* in a manner that has already been sufficiently described above. The first chaetigerous somite is therefore, strictly speaking, the second body-somite and not the third as Gamble & Ashworth suppose (1900). It is highly improbable that differences in this respect should prevail among the different species of *Arenicola*: at all events in *A. cristata*, study of the larval stages proves beyond a doubt that the composition of the anterior region is as above described.

The position of the first nephridium in *A. cristata* (body somite VI, or 5th chaetigerous somite) agrees with that of the majority of other species hitherto investigated. In *A. marina*, however, the first nephridium occupies somite V (4th chaetigerous), and its nephrostome perforates the third diaphragm and opens into the body-cavity beyond. It is noteworthy, as Gamble & Ashworth have emphasized, that this nephridium is typically smaller than the others and frequently much reduced or even absent. Since the larvae of *A. cristata* possess pronephridia in both somites V and IV, it is evident that the present condition has been reached through the disappearance of certain more anteriorly situated nephridia; and the process of reduction is apparently still in progress in *A. marina*. The formation of the anterior diaphragms is possibly in part responsible for the disappearance of the nephridia of this region, since the nephridia are thus cut off from the genital region and deprived of one of their most characteristic functions, that of conveying the genital products to the exterior.

In anatomical structure the nephridia of *A. cristata* are very similar to those of *A. marina*. The large conspicuous nephrostome with its fringed dorsal lip transversed by the nephrostomial blood-vessel, the spacious and uncoiled glandular region, and the large contractile terminal vesicle are characters apparently possessed in common by all species of the genus. The nephrostomial blood-vessel, after leaving the posterior margin of the dorsal lip, passes along the body of the nephridium and is associated in body-somites VII to X with the formation of the gonads. These organs have the characteristic form of strands of cellular tissue traversed by the

blood-vessel and formed by a proliferation of the peritoneal covering of the latter, in a manner later to be described. The position of the anterior margin of the nephrostome corresponds to the boundary between two successive somites; in early larval life the nephrostome actually perforates the septum and opens in the typical manner into the body cavity beyond. In adult life no septa are present in the nephridial region; these structures are, however, represented by the segmental blood-vessels which pass between the subintestinal vessel and the lateral longitudinal vessels. These vessels, with which the nephrostomial blood-vessels are directly continuous, are originally formed in the intersegmental septa, as already described; and in this sense each nephrostome may be considered to open throughout life into the next anterior somite. The external opening of the nephridium is at the posterior extremity of the somite, dorsal and a little posterior to the row of ventral hooked setae.

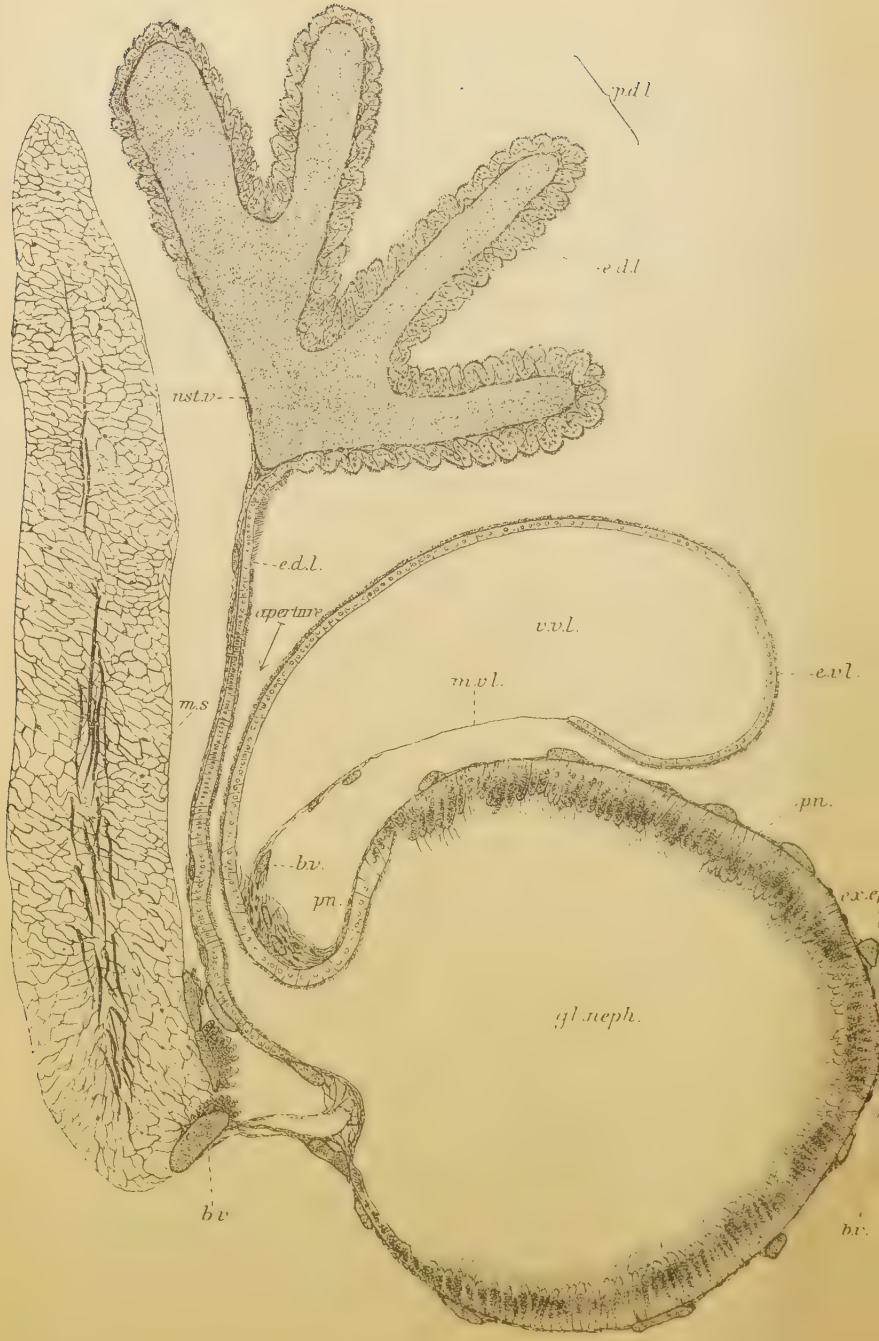
In its natural position, each nephridium is situated immediately within the dorsal insertion of the transverse muscular bands, by which it is partially isolated from the general body-cavity. When these muscles are cut and turned back, the nephridia are freely exposed to view (Plate 22, Fig. 1). Each is then seen as an elongated tubular organ with brown walls, terminated posteriorly at the level of the setae by the contractile vesicle, and anteriorly by the large and characteristic nephrostome, conspicuous from the bright red processes of its dorsal lip. A slip of transverse muscle is attached to the anterior portion of the dorsal lip and in life binds the nephrostome down in such a manner that its opening is directed inward. Figure 3 represents a nephridium seen in its natural position from above, after the removal of all the transverse musculature, except a portion of the attached slip.

The nephrostome is of peculiar and complex structure and presents certain peculiarities that apparently have not hitherto been observed in the other species of *Arenicola*. Figure 2, which represents an enlarged view of the first nephridium of the right side, shows the appearance of the nephrostome after its attachments have been removed, and its aperture has been freely exposed to view and spread open. It will be observed that a well defined line of division separates the light-colored non-pigmented nephrostome from the deep brown glandular portion of the organ, a division corresponding to a marked difference in the histological character of the lining epithelium of the two regions.

The limits of the nephrostome as such are thus sharply defined in the adult. In early stages, on the other hand, no sharp line of demarcation can be drawn. The above difference of structure must not therefore, as some suppose, be held to indicate that the two regions represent genetically distinct structures which have secondarily entered into intimate association. It will be shown beyond that the two portions develop in direct continuity with one another as portions of an originally single embryonic rudiment.

The dorsal lip is bordered by a series of bright red vascular ciliated processes. These are flattened and somewhat leaf-shaped structures, partially subdivided by secondary finger-like processes, and arranged in a single row from end to end of the dorsal lip. The dorsal lip is somewhat the longer of the two, and is usually somewhat folded in the living *Arenicola*, so that the one-rowed arrangement is not obvious on casual examination. When the nephrostome is slightly stretched, however (as in Fig. 2), the processes are readily seen to have the above-described arrangement, with their broad faces opposed like the leaves of a book. At the anterior and posterior limits of the lip they diminish in size and the number of subdivisions becomes less. Each process may be described as palmate in shape; at its somewhat narrowed base it is attached to the border of the dorsal lip; in structure it is essentially a hollow thin-walled flattened vesicle, lined internally by a connective tissue basement membrane and covered by a columnar ciliated epithelium which passes without transition into the epithelium lining the interior of the nephrostome.

The relation of the processes to the nephrostomial vessel is in reality very simple, and may be readily understood by a reference to the textfigure (p. 362), which represents in a somewhat simplified form a cross section of the entire anterior region of the nephridium. The main portion of the vessel (*nst.v.*) runs along the outer margin of the dorsal lip immediately below the insertion of the processes, and its interior communicates directly with that of the processes, whose bright red color is thus due to the contained blood. No distinction, however, can be seen between the wall of the blood-vessel and the internal connective tissue lining or basement-membrane of the processes, the two being directly continuous with one another, and in reality differing only in their relations to the nephrostomial epithelium. This epithelium is intimately related to that part of the blood-vessel which enters the interior of the processes.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Externally, however, the blood-vessel is not covered by epithelium, but merely by the investing peritoneum of the nephridium (textfig. . The relations may, therefore be described as follows: the blood-vessel is bordered by a regular series of short flattened palmate branches or folds, which are covered by the ciliated epithelium of the nephrostome and together with this form the ciliated processes of the dorsal lip.

The manner in which these peculiar relations are established becomes clearer on considering the condition in the later larval stages. The nephrostome in larvae of from 2 to 10 mm. in length is composed of cubical ciliated cells of small size; the margin of the dorsal lip is entire, and along the outer surface runs the early nephrostomial blood-vessel which at this stage is of uniform diameter throughout its length (Plate 25, Figs. 43, 44, 46—49, *nst.b.v.*). The vessel retains this position throughout life: in later stages, however, the portion of the nephrostomial epithelium in contact with the blood-vessels seems to undergo a great increase of surface and to become thrown into folds, which eventually exhibit a typically regular arrangement and definite structure and constitute the processes of the dorsal lip. The wall of the blood-vessel preserves its contact with the nephrostomial epithelium and takes part in the folding process, and hence the interior of the processes necessarily remains in continuity with the lumen of the blood-vessel. The exact manner of the transformation has not been observed, and the above provisional account is largely suppositional, since the margin of the epithelium remains entire in larvae of 10—15 mm. length, the largest I have as yet succeeded in rearing. In other respects, however, the nephrostome of this stage has essentially the same characteristics as that of the adult, and the formation of a system of folds in the manner above outlined would complete the resemblance to the adult nephridium. Gamble & Ashworth (1898) have

Explanation of Text-figure.

Somewhat simplified cross section through the nephrostome and adjoining glandular region of the adult nephridium. Section blanc passes about midway between anterior and posterior borders of the nephrostome (see Plate 22, Fig. 2). The figure shows the relations of ciliated processes of dorsal lip *p.d.l.*, nephrostomial blood-vessel *nst.v.*, vesicle of ventral lip *v.c.l.*, and glandular region *gl.neph.*. The relations of the nephrostomial epithelium to the processes and to the vesicle are shown; also those of the membranous wall of the vesicle *m.v.l.* to the epithelium *e.v.l.* and to the peritoneum *p.m.*. *b.v.*: blood-vessels; *m.s.*: muscular slip attached to dorsal lip; *ex.ep.*: excretory epithelium.

published drawings of nephridia of young specimens of *A. marina*, respectively 30 and 44 mm. in length (Figs. 16, 18), in which the dorsal processes are just beginning their formation in a manner that corresponds substantially with the above description. In *A. cristata* it is probable that they arise at a corresponding stage and in the same manner. It is interesting to note that in certain other species, notably *A. Grubii* and *A. ecaudata*, the processes, while evidently bearing the same relation to the nephrostomial blood-vessel, are fewer in number and simpler in structure, remaining apparently in a less differentiated condition throughout life (Gamble & Ashworth 1900).

All indications therefore point to the conclusion that the processes represent simply an elaborate and regular system of folds of the nephrostomial epithelium. The correctness of this view is further indicated by the identity in structure of the ciliated cells of the processes and of the other portions of the nephrostome.

By the formation of the processes of the dorsal lip, the ciliated surface of the nephrostome is enormously extended and its efficiency as an organ for removing suspended solid particles from the coelomic fluid is correspondingly increased. The cilia which cover the processes are incessantly and vigorously active (a condition presumably favored by the abundant vascular supply), and tend to sweep all suspended solid particles into the interior of the nephridium. In living Arenicolae it is found that the interior of the nephrostome and the interstices between the processes are occupied by a mass of loosely granular substance evidently collected in this way. On microscopical examination the mass is found to consist largely of broken-down cells containing excretory granules similar to those found in the chloragogen cells coating the ventral blood-vessel. It appears probable, in fact, that the chloragogen cells undergo a continual process of disintegration and that the solid excretory products thus set free in the coelom are swept into the interior of the nephridia by the nephrostomial cilia and so conveyed to the exterior. The broken-down chloragogen cells are possibly replaced by wandering cells or, more specifically, excretophores, which are chemotropically attracted to the ventral blood-vessel and while there extract from the blood the excretory matters; the latter being deposited in a solid form in the interior of the cells and eventually set free in the coelom to be removed to the exterior by the nephridia. Urea and other soluble waste-products seem, on the

other hand, to be excreted by the glandular epithelium of the nephridia. Further evidence of the excretory function of the chloragogen cells will be presented later. At present it is sufficient to note that an important part of the excretory function is apparently performed in the above manner; and it seems possible that the increased ciliary surface provided by the vascular nephrostomial processes has proved advantageous in facilitating the removal of solid excretory particles from the coelomic fluid, and has therefore been acquired in relation to this function (compare Rosa, 1903).

The ventral lip of the nephrostome differs from the dorsal lip in being entire and non-vascular, as in apparently all other species of *Arenicola*. It presents a curious and hitherto undescribed modification in the shape of a large, thin-walled, hollow vesicle (Plate 22, Figs. 2, 4) which occupies its median portion and is prolonged at either end into a tubular region of narrower calibre forming the remainder of the margin of the ventral lip. The entire ventral margin between the anterior and posterior limits of the dorsal lip is thus in reality a thin-walled tube, with a central dilatation, constituting the vesicle in question. The greater part of the wall of the vesicle and of its tubular prolongation is formed of a ciliated epithelium continuous with that lining the interior of the nephrostome (see p. 362). In cross section, the epithelium of the ventral lip is found to curve outward as the margin is reached, and to pass without demarcation into the epithelium covering the outer surface of the tubular region. This epithelium extends around the greater part of the circumference of the vesicle and the marginal tubule; it ceases abruptly, however, at a well defined line on the outer surface (Figs. 2, 6) and the remainder of the circumference is formed of a thin, translucent membrane which at its junction with the wall of the nephrostome becomes continuous with the peritoneal covering of the nephridium. Near its line of junction with the nephrostomial wall, the membrane may show a few blood-vessels derived from the general nephridial network, but towards its junction with the epithelium such vessels are generally wanting.

The interior of the vesicle is typically completely shut off from direct communication with the body-cavity. Occasionally, however, a few ova may be seen within; these may have gained access to its interior as oögonia, and have there undergone further growth and maturation. The thin-walled membranous portion may possibly

exhibit occasional interruptions in its continuity which might admit of the passage of minute bodies such as the early oögonia into the interior of the vesicle. Leucocytes appear always to be present there to a greater or less degree. Definite openings however, placing its interior in free communication with the body-cavity, do not exist. The cavity in fact appears simply to represent an enlarged lymph-space. It is lined by a thin layer of connective tissue, containing minute blood-vessels and occasional nuclei, and directly continuous with the membranous portion of the wall of the vesicle.

The remainder of the ventral lip below the tubular margin is, like all the non-marginal portion of the nephrostomial wall, composed of (1) an internal layer of cubical ciliated epithelium, lining the interior of the nephrostome; and (2) an external connective tissue layer bearing blood-vessels and continuous with the peritoneum covering the outer surface of the entire organ.

The manner in which the ventral lip acquires its characteristic structure is at present somewhat uncertain. The vesicle and marginal tube make their appearance at a relatively late period of development and are not present in larvae of 10—15 mm., the largest I have so far reared. The probable manner of their formation can, however, be inferred from a comparison of the adult and larval nephrostomes. In larvae of the above dimensions the nephrostome is formed of a single layer of cubical ciliated cells covered externally by a thin peritoneal layer (Plate 25, Figs. 43, 44, 48 etc. and possesses an entire margin, along whose dorsal portion runs the nephrostomial vessel (Figs. 42—49). The ventral lip is shorter than the dorsal lip, but in other respects possesses a similar structure (Fig. 47). The formation of a tubular margin had its origin, in all probability, primarily in an increase in the extent of the ciliated surface. In the adult organ the original border of the nephrostome is undoubtedly represented by the line of separation between the epithelial and membranous portions of the tubular margin (Plate 22, Fig. 6). The membranous portion apparently represents the original peritoneal layer, which is here no longer (as originally) in immediate contact with the outer wall of the nephrostome, but has become separated from the latter by the formation of a marginal lymph space of very definite form. The manner in which the present conditions arose was thus very probably somewhat as follows: — The epithelium of the ventral lip underwent a process of extension in an outward direction; if the area of the epithelium increased

more rapidly than that of the associated peritoneum on its outer side, the former would soon exceed the latter in extent and as a result would tend at first to curve outward and eventually, with the continued growth, to become separated from the peritoneal layer. A marginal space would thus be formed between the epithelium and the peritoneum which was originally in contact with it. This space might later acquire the definite characteristics presented by the tubular marginal space of the adult nephrostome. This space indeed, presents many of the characteristics that we should expect to find were such its mode of formation. In cross section, its epithelial wall is much more extensive than its membranous wall; its epithelium is continuous with that of the interior of the nephrostome; its interior is closed on all sides toward the body-cavity; and it is lined internally by a thin layer of connective tissue, continuous with that which is everywhere present between the peritoneal epithelium and the outer ends of the epithelial cells of the nephridium. Whether or not the conditions of its formation were of the mechanical kind above imagined, there seems little doubt that it represents essentially a large and well defined lymph space between the peritoneum and the outer surface of the nephrostomial epithelium.

The body of the nephridium is of a deep brown color; it is spacious and uncoiled, as in all other species of *Arenicola*, and tapers slightly towards its posterior termination where it joins the large contractile terminal vesicle. The brown color of these regions, like that of the external integument, is due to the numerous excretory granules contained in their walls. The walls of the terminal vesicle are thinner than those of the tubular region, and are typically folded in such a manner as to present a somewhat morula-like appearance, as represented in Plate 22, Figs. 1—4. This appearance is an incidental result of the network-like arrangement of the muscle-fibres which occupy the position of the interspaces between the elevations of the surface. Each inter-muscular area thus tends, in the contracted condition of the vesicle, to project beyond the general surface in the manner represented. Both nephridial tube and terminal vesicle are richly supplied with blood-vessels.

Histology.

The lips of the nephrostome and its interior are lined by a single layer of columnar ciliated cells which show but slight differences of structure in the different regions. Over the surface of the

dorsal processes, the epithelium is folded (Text-figure on p. 362) and is composed of cells of a more columnar shape than those covering the ventral lip, which are more cubical in appearance (Plate 22, Fig. 6). The difference in shape is, however, inessential and is due in all probability to the different conditions of pressure and tension to which the cells are exposed in the different regions; the epithelial cells of the processes — being apparently subjected to lateral pressure, as indicated by the folding — are for this reason, more columnar; while the cells of the smooth unfolded epithelium of the ventral vesicle are apparently subjected to the tangential tension characteristic of distended vesicles, and are thus forced to assume a more cubical shape. In all essential respects, however, the cells are identical in structure. They are large and clear, of approximately the same size in different regions, and do not contain concretions. Each is bordered by numerous strong cilia (each of which has a well-defined deeply staining basal granule), and the protoplasm exhibits in stained preparations the fibrillar structure characteristic of ciliated cells. As Gamble & Ashworth have pointed out (1900, p. 515) the cells are much larger than those of the cubical epithelium of the early nephrostomes. The relative size of the two may be seen by comparing Plate 22, Fig. 6 and Plate 25, Fig. 47, which are drawn to the same scale. It will be seen, however, that the nuclei of the early nephrostome are of almost exactly the same size as the nuclei of the adult structure, and occupy a correspondingly large portion of the cell. In their ciliation, character of the protoplasm, and general shape, however, the cells are closely similar.

The cubical epithelium of the funnel passes over into the excretory epithelium of the glandular region by a somewhat abrupt transition, although a few of the cubical cells immediately adjoining the excretory epithelium proper may contain granules in their interior and in other respects exhibit a partially intermediate structure.

The large excretory cells of the glandular portion of the nephridium form a single layered excretory epithelium lining the interior of the organ and generally thrown into a well marked system of internal folds. External to the epithelium is a thin connective tissue layer bearing numerous blood-vessels; externally the whole organ is covered by the peritoneum.

In structure the cells agree closely with those of *A. vecaudata* as described by Gamble & Ashworth. Like most cells of an

excretory function they are much vacuolated and contain numerous deeply staining granules. The nucleus is situated in the middle region of the cell, usually somewhat nearer the base than the outer border. It is large, generally rounded in shape but frequently of irregular outline, and contains a chromatin network of the appearance represented in Plate 22, Fig. 5, together with a conspicuous deeply staining nucleolus which in nearly every instance is seen to contain a well-defined vacuole. The vacuolation of the nucleolus is characteristic of the excretory cells, and also, as will be pointed out later, of the large nuclei of the growing zone, and of the glandular cells of the ventral wall of the stomach. The peculiarity seems to be associated with unusual metabolic activity. It is noteworthy that in the excretory cells the position of the nucleus always closely corresponds with that of the zone of deeply staining excretory granules. — The different regions of the cell show very constant and characteristic peculiarities, as may be seen from Fig. 5 which represents a portion of the excretory epithelium seen in cross section. At its base each cell shows typically a denser structure than at the more peripheral portions, and in fixed and stained preparations, seems composed chiefly of a feltwork of prevailingly longitudinal fibrils — an appearance which seems characteristic of ciliated cells. Typically the basal part of the cell is almost free of granules. Towards the middle region where the nucleus is situated, the protoplasm becomes more vacuolated and is in most instances largely filled with minute round granules which stain an intense black with iron-haematoxylin. Besides these deeply staining granules, there are others (which in some cells may preponderate: see Fig. 5) which stain less deeply and seem to have a greater affinity for the acid stains such as erythrosin. The restriction of the deeply staining granules to the middle region of the cell is very constant, though not absolutely so, and gives rise in cross section to the appearance represented in the text-figure (p. 362). The number of granules, however, shows great variability, some cells being almost free of them while others are so deeply laden as to present an almost uniformly black appearance. The extreme outer portion of the cell is usually, but not always, free of black granules, and is composed of a finely granular, much vacuolated protoplasm which frequently contain numerous lightly staining rounded bodies, apparently of the same nature as the erythrophilous granules just described as present in the middle region of the cell. These bodies range in size from

minute granules to large rounded masses of half the diameter of the cell; they are of very variable occurrence, at times very numerous and at other times almost entirely absent. They apparently represent the final stage of the excretory products immediately before their extrusion into the lumen of the nephridium. Frequently bodies of precisely similar appearance are found in the lumen immediately without the cell wall and apparently still adhering to the latter. Such bodies have evidently recently been extruded. The indications are therefore that the black granules do not represent the final stage of the excretory products; but that the latter undergo a further chemical transformation in the outer portion of the cells before being cast out into the lumen.

The outer rounded margin of the cell usually projects into the lumen of the nephridium and bears flagella. The number of flagella is not, however, constantly one to each cell, as sometimes held; a single cell may frequently bear a tuft composed of several flagella. The projection of the cell-body into the lumen is in general less prominent in cells that contain relatively few excretory granules.

The terminal vesicle is also lined internally by excretory cells of the same general character as the above. Between the bases of the epithelial cells and the investing connective tissue layer, is situated a strong network of muscle fibres whose strands divide the surface of the vesicle into the hemispherical elevated areas above described. In other respects, the histological structure of the vesicle is similar to that of the tubular region. The muscular network is derived, in a manner to be later described, from the longitudinal fibres of the body-wall and is continuous with these.

A comparison of the excretory cells of the adult nephridium with those of earlier stages shows various points of agreement and disagreement. Fig. 6, Plate 22 shows a longitudinal section through the wall of the nephridium of a larva of 5—6 mm. The cells, it will be observed, are much shorter and broader than those of the adult nephridium and contain fewer granules, which furthermore fail to show the definiteness of distribution found in adult individuals. In the general characteristics of the protoplasm, its vacuolation, and its greater density at the base of the cell, the larval excretory cells agree with the adult; and the nuclei are of almost precisely similar size and appearance, even to the presence in each of a conspicuous nucleolus which almost always contains a well defined vacuole. The

vascular supply of the young nephridium is much less developed than in the adult; and the cells of the terminal vesicle are flatter and contain fewer granules, while the muscular layer of its wall is largely incomplete (Plate 25, Figs. 45, 55).

The number of granules in the excretory cells of larval stages is however subject to great variation, as will be seen by a reference to Figs. 46—54 which represent cross sections from the first nephridium of a single specimen of somewhat smaller size than the one from which Fig. 7 was taken. In this specimen the nephridial cells contain numerous and characteristic excretory granules; in other respects, however, they are essentially similar to those of the first specimen. The projection of the individual cells into the lumen is present here as in later stages, though it is less marked.

Later the histology of the larval nephridia will be dealt with in more detail.

5. Development of the Nephridia.

The nephridia develop in close relation to the other segmental structures of the body, especially the septa with which they are from the first in direct continuity. The early somites, as in other Annelids, make their appearance as immediate products of the activity of a growing zone, comparable with the teloblastic region of leech or oligochaete embryos. It will be necessary therefore, in order to make clear the earliest relations of the nephridia, to consider in greater detail the characteristics of this zone and of the region of active differentiation immediately anterior to it.

(a) Growing Zone.

The appearance of the growing zone in longitudinal section is represented for early larvae in Plates 22 and 23, Figs. 7, 8, 9, 10; for somewhat later stages in Plates 23 and 24, Figs. 30—32; and for the stage of formation of the achaetous body-region in Plate 24, Fig. 33. Its appearance in cross section may be seen from Figs. 14, 21 and 24, Plate 23. Its characteristics remain almost constant for the whole period of its activity; after the formation of 56—58 somites it disappears and the formation of somites ceases. Throughout the whole period of somite-formation the zone retains a constant position a short distance in front of the posterior extremity of the body, immediately anterior to the post-mesodermal pygidial or former paratrochal region. Posteriorly where it adjoins the pygidial region its

boundary is sharply defined; anteriorly it passes by a gradual transition into the more fully differentiated region in front. The pygidial region thus takes no part in the formation of somites, but is carried passively backwards as the body elongates and preserves unchanged its peculiar characters throughout the whole development.

The histological structure of the growing zone may be seen by a reference to the figures above mentioned. Cross sections show that the ectoderm of the extreme posterior region of the body of the young larvae is greatly thickened, and of almost uniform diameter in all portions of its circumference (Figs. 14, 21 and 24, Plate 23). Figure 14 is a section passing through the posterior region of a larva of 5—6 somites. The section is slightly oblique; dorsally it cuts through the anterior portion of the paratrochal region, and ventrally through the region of commencing differentiation at the posterior border of somite V. The ectoderm of the growing zone is seen to be composed of a single layer of large cubical cells containing large clear nuclei, each of which usually exhibits a well-defined nucleolus. On passing forward from this zone the dorsal and lateral regions of the ectoderm are seen gradually to become thinner, the cell-limits become less sharply defined, the number of nuclei in cross section becomes fewer, and the nuclei themselves become smaller and more deeply stained, gradually assuming the usual characteristics of the tissue-nuclei. The ventral ectoderm also becomes thinner anteriorly, although to a less extent than the dorsal and lateral regions; and the ventral nerve-cord appears as a fibrillar differentiation of the inner ends of its cells.

The region of large cells with the large clear nuclei thus evidently corresponds to the undifferentiated embryonic region of the growing zone, from which cells are divided off anteriorly: in other words, to the teloblastic region. Definite individualized teloblasts however are not present in Arenicola at this stage. The number of cells in cross section is not constant and the cells show no disposition to become arranged in rows, but simply form an undifferentiated zone which passes by a gradual transition into the more differentiated ectoderm in advance. Posteriorly the ectodermal cells of the growing zone are, as already mentioned, sharply delimited from the small, radially arranged, pigmented cells of the adjoining pygidial region (Plate 24, Fig. 32, 33).

The thinning-out which the ectoderm shows on passing forward from the growing zone is in all probability due in large part to a

distension of the body-wall by the contained coelomic fluid, whose osmotic pressure must necessarily exceed that of the sea-water otherwise collapse would result. The extension of the body-wall in the region immediately anterior to the growing zone is certainly not due purely to a process of cell-multiplication or simple growth. This is shown by the fact that the wall becomes thinner and the nuclei more sparsely distributed at the same time as the superficial extent increases, indicating clearly that the material which is being added at the growing zone is undergoing a re-arrangement and extension of a kind similar to that which would result from mechanical stretching. The expansive force which in this manner stretches the newly formed body-wall and thus causes this region to extend both transversely and longitudinally (see Plates 2'—24, Figs. 9, 11, 29—32), is in all probability the osmotic pressure of the coelomic fluid. The process may be compared roughly to the formation of a soap-bubble, where the thick layer of fluid added at the rim of the pipe-bowl corresponding to the growing zone is thinned-out and extended in both directions by the pressure acting on the walls from within. The analogy need not be pursued in further detail; it is probably accurate to a certain degree, although in the growth of the larval body-wall material is undoubtedly added at other regions than at the growing zone. The analogy fails for example to explain why the ventral body-wall remains thicker than the dorsal and lateral regions. The above peculiarities of the ectoderm at and immediately in front of the growing zone are however rendered partly intelligible by this comparison.

The space between entoderm and ectoderm at the growing zone is filled with a mass of undifferentiated mesoderm; more anteriorly the mesoderm becomes differentiated and shows the usual division into somites. At the extreme posterior narrow region between the entoderm and the ventral ectoderm are found undifferentiated cells of a characteristic embryonic appearance (Plate 23, Figs. 10, 30, 31). These cells are typically larger and clearer than those situated more anteriorly, and are frequently found in process of mitosis; they possess large clear nuclei each of which typically contains a conspicuous vacuolated nucleolus. They undoubtedly correspond to the teloblasts or mesoblastic pole-cells which terminate the mesoderm bands in so many other species. In *Arenicola*, however, their number is not constant, nor are they sharply defined from the mesoblast cells immediately in front. The extreme posterior mesoderm

might indeed be more accurately described as consisting of a continuous mass of undifferentiated protoplasm, containing large nuclei from which smaller nuclei are divided off anteriorly to form the nuclei of the differentiating mesoderm. The terminal cells cannot thus strictly speaking be regarded as possessing an individuality which persists through development. Described objectively the conditions are simply as follows: the extreme posterior mesoblastic region contains large clear nuclei in process of active multiplication; the nuclei that remain in this position retain their embryonic character, while those which are given off anteriorly become smaller and more chromatic (to describe simply the change in appearance), and form the nuclei of the newly differentiating tissues. Cellular demarcations are in fact difficult or impossible to observe in the early mesoderm of *Arenicola*; the term mesenchyme is therefore perhaps more accurate in that it does not imply a definite epithelial arrangement of the cells — a condition which is never found in the growing zone of this species.

The undifferentiated portion of the mesoderm is of very limited extent (Plate 22 and 23, Figs. 9, 10, 29—31); almost immediately in front of the teloblastic region the mesenchyme cells become arranged to form the primitive septum bounding the newly forming somite; while the cells applied to the body wall in the same region show fine muscle-fibrils in their interior — the precursors of the longitudinal muscles of the body-wall (Plate 23, Figs. 13, 14, 21, 24). The limits of the somites are thus defined very early: when first formed they are very short (Plate 22—24, Figs. 9, 10, 29, 32); they soon elongate, and attain the normal proportions a short distance in advance of the growing zone.

The ectoderm and mesoderm of the growing zone thus agree in the possession of large undifferentiated cells containing large nucleolated nuclei, which form the posterior termination of the growing region and represent the teloblastic or undifferentiated embryonic region of the elongating embryo. The fact that the cells of this region are indefinite in number and arrangement — differing markedly in this respect from the sharply individualized teloblasts of *Clepsine* (Whitman, 1887) and *Lumbricus* (Wilson, 1889) — is not in any degree inconsistent with their being described as teloblastic. Whether the terminal growing region is constituted of one cell or of several cells seems indeed to be immaterial. The distinctive characteristic of teloblastic growth is the presence of a terminal

undifferentiated growing region, which retains its embryonic character throughout development and gives rise anteriorly to the differentiated tissues of the developing organism; and on this criterion the development of both ectodermal and mesodermal structures in *Arenicola* is typically teloblastic. The entoderm on the other hand does not apparently elongate by teloblastic growth and it does not, properly speaking, exhibit a metameric structure in the same sense as do the ectoderm and mesoderm. Its thin-walled posterior portion is frequently drawn out at the lines of insertion of the primitive septa (as represented in Plates 23 and 24, Figs. 10, 30—33), and in this manner partakes of the early metamerism; but this apparent metamerism seems to be purely incidental and imposed upon the organ from without, and therefore not inherent in its structure. It is noteworthy that in other species that show teloblastic growth the intestine never seems to develop teloblastically. This fact seems to afford support to those authors (such as Hatschek, Meyer, Bergh, Goodrich) who believe that metamerism in annulate animals is primarily mesoblastic, and that the other metameric systems of organs have become implicated in a purely secondary manner.

The large mesodermal and ectodermal cells of the growing zone are present, as above described, throughout the entire period of somite-formation. The ectodermal cells are evidently the lineal descendents of the cells described by Child (1900) as derived from the division of the "stemcells", — i. e. of the two symmetrical derivatives of the somatoblast (2^d) of the second quartette of ectomeres, which form the posterior portion of the somatic plate. These cells at the commencement of the concrescence of the somatic plate form "a transverse band of seven cells lying just anterior to the paratroch and extending in the same direction with it. These cells are large and thick and grow rapidly after division. It seems perfectly clear that we have here what is known as the 'growing tip', i. e. that portion of the body just anterior to the paratroch, which continually gives rise to the new segments and leads to the elongation of the larva. The final results of concrescence will be first, to unite the ends of the paratroch, and then the ends of the second row of cells — the growing tip" (p. 628). Child did not follow the concrescence to its completion; but from the position and relations of the large ectodermal cells of the larval growing region there can be no doubt that these are the direct descendents of the

above band of cells (see CHILD, loc. cit., Figs. 112, 113). The cells retain their large size throughout larval development, showing little change in character from the early larval stages (three somites) until the completion of the definitive number of somites.

The large terminal mesoblast cells of the larva are similarly to be regarded as the descendents of the two teloblasts of the early embryonic mesoderm (CHILD, loc. cit. p. 638). The embryonic mesoblast bands take up a ventro-lateral position between the entoderm and the ventral ectoderm of the posterior region (Fig. 119). At this stage each band is terminated by a single large teloblast. According to CHILD "visible differentiation in the descendents of the mesoblasts is very slow. The cells retain their embryonic appearance till a late stage" (p. 639). The original condition in which each mesoblast band is terminated posteriorly by a single teloblastic cell does not apparently persist long, for in the larval stages the teloblastic region of the mesoblast always contains several large embryonic nuclei, as above described, which exhibit no constant number or arrangement. Their position, however, between the posterior attachment of the intestine and the ventral ectoderm corresponds accurately with their position in later embryonic stages as described by CHILD. Between the latest stages described by this author and the earliest I have examined intervenes a period which has not yet been studied with reference to the character of the mesoblast. There can however be no doubt that examination would disclose a direct continuity between the teloblasts of the later embryonic stages, and the posterior undifferentiated mesoderm cells of the larval growing zone.

(b) Formation of the Somites.

The first larval nephridium appears in somite IV and becomes recognizable soon after the formation of the early septum which separates this somite from the one immediately anterior to it. Young larvae that have just terminated the swarming stage (e. g. Plate 22, Fig. 8) thus generally exhibit this nephridium in the early stages of its formation in the mesoderm of the posterior region. Since all of the nephridia arise in exactly the same manner, a description of the changes taking place in the posterior mesoderm of such a larva will apply to all of the early nephridial somites. The mesoderm of the non-nephridial somites undergoes closely similar changes —

— the mesoblastic somites of the whole larval body being indeed formed in an essentially unvarying manner.

As the larva advances in development the nephridia of the more anterior somites increase in size and undergo a more complete differentiation, at the same time as the others arise in the growing region. Horizontal sections passing through the nephridial region of such larvae may in fortunate preparations show the entire series of nephridia, from the early undifferentiated rudiments of the region adjoining the growing zone, to the well-defined nephridia of the more anterior somites. Fig. 29, Plate 23 represents such a preparation in the case of a larva of about eleven somites. On the right side the section passes slightly ventrad to the ventral hooked setae and shows the larval nephridia from the first (in somite IV) to the sixth (somite IX); the undifferentiated rudiment of the 7th (5th definitive nephridium) is just visible at the angle between the newly forming septum and the bodywall. In Figs. 16 to 22, inclusive (Plate 23), have been represented cross sections of the successive nephridial somites and the post-mesodermal region of a larva in which somite IX is in process of formation. The different stages in the early development of a nephridium can thus be studied to advantage in single larvae of this and similar stages. The development of the nephridia is however so intimately bound up with that of the somites that it will be necessary first of all to consider the latter process in detail.

The first changes, preparatory to somite-formation, that take place in the posterior undifferentiated mesodermal region consist in a multiplication and subsequent rearrangement of the mesodermal cells, which at first are tightly packed together at the posterior angle between the intestine and the body-wall (Plates 22—24, Figs. 7—10, 29—33). The manner in which this rearrangement is accomplished may be seen from the above drawings, especially the sagittal sections (Plate 23, Figs. 10 and 31), and the horizontal sections (Plate 22, Fig. 9 and Plate 24, Fig. 32). The closely packed posterior mesoderm is here seen to be bounded anteriorly by a roughly defined transverse partition whose cells are directly continuous with the mesenchyme cells lining the cavity of the more fully formed adjoining somite (VI, Fig. 9; somite VIII, Fig. 10). This partition, therefore, represents the posterior wall of the somite next in front of the one in process of formation, and defines the position of the future intersegmental septum, of which structure it in fact forms the anterior

lamella, as will be shown beyond. As the number of mesenchyme cells behind this partition increases — chiefly by the multiplication of the large polar nuclei — an irregular hollow space appears between the cells in the neighborhood of the partition, and gradually enlarges, apparently in part by the absorption of fluid from the adjoining coelomic cavities. The space so formed represents the early coelomic cavity of the somite which is in process of formation; it becomes progressively better defined as growth proceeds, while the layer of mesoblast forming its walls becomes irregularly applied to the surface of adjoining structures — anteriorly to the posterior wall of the next somite in front, externally to the ectoderm of the body-wall, internally to the wall of the intestine and posteriorly to the densely packed mesoderm of the growing region behind. The partition formed by the apposition of these mesoblast cells to the posterior wall of the somite in front represents the earliest condition of the newly forming intersegmental septum, and thus defines the anterior boundary of the newly forming somite. The early septum thus consists essentially of the opposed walls of this incipient somite and of the somite in front. Its characteristics and relations to the nephridia will shortly be considered in detail.

The bilaterality of the early mesoderm is not very decidedly pronounced. A ventral mesentery is apparently not formed in the anterior region of the body and the coelomic cavities of the two sides are therefore from the first continuous below the intestine. Dorsally, however (Plate 23, Fig. 13), the right and left coelomic cavities remain distinct, and the adjoining walls (which soon assume a membranous character) become applied to one another above the intestine, forming a bilaminar membrane, whose lamellae are from the conditions of their formation necessarily continuous with the mesoderm covering the intestinal and body-walls, and with the lamellae of the primitive septa. This membrane constitutes the dorsal mesentery which persists throughout life and suspends the intestine to the dorsal body-wall.

Before considering in greater detail the characteristics of the primitive septa, the formation of the dorsal and ventral blood-vessels may be briefly described, since these structures appear very early and apparently play an important part in the nutrition of the growing region. The dorsal blood-vessel is early formed as a space between the opposed lamellae of the dorsal mesentery (Plate 23, Figs. 13, 20 and 21). It does not, however, appear to become well

defined so soon as the ventral vessel, being possibly less necessary in early stages than the latter, in which the blood flow is from before backward towards the growing zone. This vessel, as will be seen from Fig. 9, Plate 22, early appears as a well defined channel extending almost to the posterior extremity; it arises as a space between the intestinal wall and the mesoderm and soon becomes enclosed on all sides by the extension of the mesoderm cells. Its walls are from the first continuous with the original septa, a fact of significance, since the segmental blood-vessels arise in the septa and in this manner acquire their characteristic relations to the nephridia, as will be explained beyond.

Mesoderm cells remain applied to the wall of this vessel, and as development proceeds they acquire a voluminous and vacuolated appearance, and pigment or excretory granules become deposited in their interior. These cells constitute the chloragogen cells; they are thus in reality modified peritoneal cells, which throughout life coat the ventral blood-vessel and play an important part in the process of excretion (cf. Rosa 1903). They have in most larvae already assumed their excretory character in the third somite in front of the growing zone, and from this region they may be seen to pass backwards by a gradual transition into the posterior undifferentiated mesenchyme cells (Fig. 9). Their characteristics will be considered in greater detail below.

Dorsal and ventral blood-vessels soon acquire open communication with each other in the growing zone, thus completing the circulation and providing more fully for the nutrition of this region. The nature of this communication may be seen from Fig. 24, Plate 23, which is a cross section through the posterior region of a larva of 11–12 somites, passing through the region of the primitive septum between somites XI and XII. Dorsal and ventral vessels are here seen to be connected by a blood-sinus which completely surrounds the intestine. A sinus of this nature is typically present in larvae of this and later stages; it consists of a simple space between the intestinal wall and the mesoderm, and is situated usually at the insertion of the most posterior primitive septum, but frequently (see Plate 23, Fig. 30 and Plate 24, Fig. 32) at the membrane which separates the posterior mesoderm from the space between proctodaeum and ectoderm in the post-mesodermal region. The sinus varies greatly in its size and in the amount of blood that it contains and at times seems to become obliterated. Apparently it is formed as required

at the zone where least resistance is offered to the passage of blood between the two vessels, and thus represents no sharply defined or persistent channel. The branching blood-sinus which in later life envelopes the stomach-region seems to be formed in essentially the same manner.

The primitive septa are formed, as above described, by the apposition of the adjoining walls of two successive mesoblastic somites. The walls of these early somites are, however, never formed of a regular epithelial layer of well-defined cells, but to all appearance consist of a continuous syncytial layer of protoplasm containing numerous nuclei (Plate 23, Figs. 13, 14, 23, 24, and closely applied to the surface of adjoining structures. Each nucleus may for purposes of description be regarded as belonging to a single cell, but definite cell-walls, at this stage at least, are never distinguishable.

The structure of a septum at its earliest distinguishable phase may be best seen in Figs. 9 and 10, Plate 22 and 23, and Fig. 31, Plate 23. At this period of its formation the septum forms an irregular nucleated partition, separating the cavity of the newly-forming somite from that of the somite next in advance. At this stage no bilaminar structure can with certainty be discerned in it, and the constituent cells, which resemble in all respects the other mesodermal cells of this region, seem to be entirely undifferentiated and similar to one another. As growth proceeds, however, the coelomic cavities increase in size and in transverse diameter in the manner described on page 374; the septa are thus subjected to a stretching process and increase rapidly in superficial extent, becoming at the same time thinner and better defined. During this process the opposed faces of the cells of the two septal layers (which are now usually distinguishable from each other assume a more membranous character, becoming at the same time more deeply staining and apparently of a denser consistency (Plate 24, Fig. 32). As the septum continues to extend it acquires more and more of the character of a thin membrane, and the nuclei of its constituent cells are moved farther and farther apart. Finally the septum acquires the appearance of a thin, sharply defined, membranous partition extending across the body cavity, and to whose surface the original nuclei (each of which is still surrounded by a small quantity of undifferentiated protoplasm) are applied at infrequent intervals (Plates 22—24, Figs. 9, 29—34).

At the junction of the septum with the body-wall and with the

subintestinal blood-vessel the two closely applied lamellae are usually seen to be slightly separated from each other where they pass into continuity with the peritoneal membrane. In the larva represented in section in Fig. 9 the interlamellar space is continued for a slight distance outward from the lumen of the sub-intestinal blood-vessel; this space represents in all probability the beginning of the segmental blood-vessel which later passes to the body-wall and traverses the dorsal lip of the nephrostome. The septum is seen in this figure (and also in Fig. 10, 29, 31, 33, Plates 23 and 24) to become continuous at its lateral margin with the lips of the nephrostome. At its insertion into the body-wall its cells become continuous with those lining the body-cavity.

The line along which the septum is inserted into the body-wall is usually considered to mark the boundary between two successive somites. This line occupies in *Arenicola* a constant and definite position with reference to the other segmental structures of the developing larva. During the formation of the mesoblastic somites the body-wall also undergoes a division into segmental regions. The metameric structure of the ectoderm is most conspicuously indicated by the setae which appear at an early stage in the intervals between the successive septa. The seta-sacs which give rise to these structures appear laterally in the ectoderm in a position slightly behind the middle of the interseptal region (Fig. 32, Plate 24). The ventral ectoderm in the neighborhood of the nerve-cord also early exhibits a metameric structure, becoming slightly thinner or constricted at segmental intervals. The septa are inserted ventrally along a transverse line passing immediately in front of the constriction (Plate 23, Fig. 30). This line, therefore, if the above criterion of segmental limits is accepted, marks the boundary between successive somites. Later in development, when the septum has disappeared as such, the position of the line of demarcation corresponds closely with that of the nephrostome and its associated branch of the segmental blood-vessel — both of which structures, as already stated, arise in intimate connection with the primitive intersegmental septum.

The septum, when first formed, constitutes a complete partition between the body-cavities of adjacent somites. Soon after its formation, however, it becomes incomplete dorsally, as already mentioned (p. 349), and for the remainder of its existence it is found only in the ventral portion of the body-cavity as an incomplete membranous

partition, extending from the ventral blood-vessel to the ventro-lateral body-wall. It usually becomes incomplete at the second or third somite in advance of the growing region (Plates 22 and 23, Figs. 8—10, 29, 30), apparently as a result of the thinning and eventual disruption of its dorsal portions. The conditions that lead to this result seem to depend in part upon the progressive increase in the transverse diameter of this region of the body, associated, as above described, with an increase in the extent of the body-wall. A further reference to Plate 23, Figs. 16—21, which represent sections through successive somites of a single larva, will show that the increase in transverse diameter is dependent chiefly upon the extension of the thin-walled dorsal and lateral regions of the body-wall. It seems, therefore, probable, as above suggested, that the septa first become incomplete in the dorsal region largely in consequence of the undue stretching to which they are there subjected, which has caused here a thinning and eventually a disruption and disappearance of the septa. On the other hand, their ventral portions persist for a very considerable period, and play an important part in later development in connection with the nephridia and nephrostomial blood-vessels.

(c) Formation of the Nephridia.

I shall now proceed to a description of the histogenetic processes by which the nephridia are differentiated out of the originally uniform somatopleuric layer of the mesoblast. In the region immediately adjoining the pole-cells this layer, as described above, is composed of a continuous nucleated protoplasm without cell-boundaries and without visible differentiation; at a slightly more anterior position, however, where the somatopleure passes into continuity with the newly forming septum, the differentiation of the longitudinal muscle-fibres of the body-wall is already in progress. These structures make their first appearance at a very early period, in that portion of the mesoblast which is in immediate contact with the ectoderm of the body-wall (Plate 23, Figs. 13, 14, 21, 24). The fibres first become visible in two ventro-lateral areas on either side of the ventral nerve-cord, which is just beginning its appearance (*n.c.*); and at a slightly later period they appear also in two dorso-lateral areas on either side of the dorsal mesentery. Along the line of insertion of the dorsal setae muscle-fibres do not at first appear.

At the earliest appearance the fibres have in cross section the appearance of a row of deeply-staining dots at the boundary between ectoderm and mesoderm (Plate 23, Figs. 13, 21, 24). At a slightly later stage (Figs. 15, 23 the dots have become larger and better defined, and have assumed the typical appearance of triangular wedge-shaped structures, which stain very deeply in the erythrosine and project into the protoplasm of the mesoblast cells. In the ventro-lateral region the mesoblast nuclei have become arranged in a single layer, and in cross section the mesoblast has assumed the appearance, which is very typical for this stage, of a row of nucleated cells, unseparated by cell-walls, and containing in their interior deeply staining longitudinal muscle fibrils Plate 23, Figs. 13, 15, 19, 23). It is evident that the longitudinal muscle-fibres are originally laid down in the interior of these cells, which may therefore with propriety be termed myoblast cells. The cells, however, it is to be noted, present no constant and regular arrangement and are not sharply defined from one another. At this stage the longitudinal muscles are thus composed of fibrillae applied to the body-wall and covered internally by a nucleated layer of undifferentiated protoplasm (sarcoplasm). The condition recalls that which exists throughout life in certain other species, particularly the Nemathelminthes. The transverse muscles on the other hand are not visible until a much later stage; they appear externally to the longitudinal muscles, and apparently in relation to the inner layer of the ectodermal cells[1]. The above myoblasts at all events have nothing to do with their formation.

Cross sections passing through the region immediately behind the early septum show the above-described appearances in a characteristic manner (Figs. 13, 15, 19, 23). Longitudinal sections (Plates 22 and 23, Figs. 9, 10 and 31), passing through the ventro-lateral regions, show that the mesoblastic layer is thickest and best defined at the junction of septum and body-wall. It is in this region that the embryonic rudiments of the nephridia (*n*) first become distinguishable from the rest of the mesoblastic layer. It must be emphasized however, that there is at first no setting aside of special nephridial cells; nothing comparable to the septal nephroblasts or "funnel-cells" described by

[1] Ed. Meyer 1901 gives a summary of the evidence on this point, from which it would appear that an ectodermal origin is the rule, if not invariable, for the circular body-musculature of annelids.

Vejdovsky, Wilson and Bergh and more recently by Bürger (1902). The nephridia simply become gradually differentiated out of an originally uniform and homogenous mesoblast which in early stages presents no definite cell-boundaries. The differentiation of the nephridium starts from the angle between septum and body-wall; but at first no visible differences exist between the nephroblastic mesoderm and the adjoining myoblastic or septal mesoderm.

The first signs of the appearance of the nephridium are visible usually at the second septum in advance of the growing zone Young larvae of five somites thus as a rule exhibit the first larval nephridium in the earliest stages of its formation. The longitudinal section represented in Fig. 9, Plate 22 shows the differentiating 2d nephridium in somite V. At this stage the rudimentary organ has the form of a somewhat triangular mass of protoplasm continuous anteriorly with the fourth septum. Fig. 10, Plate 23 shows a similar stage in the formation of the fourth nephridium; and Fig. 31 similarly represents the rudiment of the sixth. The continuity with the septum at the junction of the latter with the body-wall is perhaps the most characteristic feature of the early nephridial rudiment.

The histological differentiation of the nephridial cells can be studied to better advantage in transverse sections. In Plate 23, Fig. 13, which represents a section through the fourth septal region of a larva of 5—6 somites, the somatopleuric cells of the ventrolateral region are entirely alike, with nothing to distinguish the future nephridial region from the other portions of the mesoblast. The protoplasm is homogenous and non-granular, the nuclei are similar to one another in size and appearance, and cell-walls are indistinguishable. At this stage in fact no essential differences exist between the cells of this intersegmental region, and those of the intersegmental region of somites II and III, where no nephridium is formed (Plate 23, Fig. 12). In Fig. 15, which represents a section through the beginning of somite VI in a larva of about eight somites, one of the cells of the right side is distinguished from the others by the presence of a small space, bordered by deeply staining protoplasm, which has made its appearance in the immediate vicinity of the nucleus. This minute space is the first indication of the appearance of the nephridial lumen, which, it is of interest to note, always seems to arise in the neighborhood of the nucleus. In other respects the cell which shows this peculiarity is entirely similar to the adjoining purely myoblastic cells, even to the possession

of muscle-fibres in its interior. Fig. 23, Plate 23 shows a similar condition. The nephridium is thus differentiated out of a portion of the original mesoblast which in its early stages possesses the same properties and undergoes the same transformations as the other portions of the layer.

At a slightly later stage the nephridial cells become more readily distinguishable from the adjoining purely myoblast cells. The lumen becomes more distinct and the body of the differentiating organ increases in size, apparently in part at the expense of the protoplasm of the adjoining mesoblast cells, which become visibly smaller. In cross section the nephridial rudiment is soon observed to be more prominent than the adjoining myoblastic cells, and its boundaries appear better defined (Plate 23, Figs. 23 left, 19 left). Its protoplasm becomes vacuolated and minute granules in many cases begin to make their appearance in its interior; the lumen becomes progressively wider and better defined and the cilia become visible. In the section represented in Fig. 19 (3 somites from the growing zone) the nephridium of the left side is somewhat more advanced than that of the right and is becoming sharply defined from the adjoining myoblast cells, which have perceptibly diminished in volume. In the next somite of the same larva (Fig. 18) the boundaries of the nephridium are quite definite, and flattened mesoblast cells are becoming applied to its surface to form the outer peritoneal covering of the organ. The nephridium in the somite next in advance (Fig. 17) is still more highly differentiated; the lumen is more spacious and the excretory granules are more numerous. At this stage the organ has the form of a simple tubule with an intracellular lumen opening anteriorly through the septum into the cavity of the adjoining somite. The progressive histological differentiation of the early nephridia is well shown in the series of sections represented in these drawings (Plate 23, Figs. 16—21).

As the nephridium advances in development the myoblast cells gradually diminish in volume, while at the same time the muscle-fibrils in their interior increase in size (Figs. 16—21). Eventually the undifferentiated protoplasm or sarcoplasm is reduced to a thin layer occupying the interstices between the fibrils and containing here and there a flattened nucleus. In later stages (Plate 23, Figs. 25—28) the muscle fibres become much more numerous and more closely set together, and the sarcoplasm is reduced to an inconspicuous interfibrillar matrix in which the fibres are imbedded. The innermost layer

of the sarcoplasm seems to become membranous and to contribute to the formation of the peritoneal lining of the body-cavity.

The nephridial rudiment can as a rule be distinguished before the septum has assumed a definite membranous structure (Plate 23, Fig. 31). It is therefore difficult to decide whether or not the nephridium is derived chiefly from one or other of the two lamellae of which the septum is composed, or from both. From all appearances, however, it seems clear that neither lamella is exclusively concerned in its formation but that both contribute in part; and that the post-septal mesoblast also participates in the process. The protoplasm of the nephridial rudiment and that of the septal cells are at first continous with one another; it is only later that the boundaries of the two become sharply defined. The fact, however, that at its very earliest appearance the nephridial lumen communicates with the body-cavity of the preceding somite proves that the mesoblastic wall of this somite enters, in part at least, into the formation of the early nephrostome. It cannot, however, be said that the nephrostome is formed exclusively from the posterior wall of this somite. The early nephridial rudiment is in fact a continuous and undivided structure without regional delimitations, and it is not until much later that the nephrostome becomes distinct from the glandular portion of the organ. It is impossible, therefore, to say that one definite portion of the nephridium is derived from one somite and another portion from another somite. It is even impossible in very early stages to sharply define the limits of the mesoderm of successive somites, since the differentiation of somites is itself a gradual process, and the nephridial rudiment is already laid down before the segmental limits are sharply defined. It is, however, true that in its early undifferentiated condition the nephridium is continuous with mesodermal tissue which later forms portions of two successive somites. The organ, however, is differentiated as a whole without strict reference to segmental limits. Even cell-limits, as already pointed out, are not sharply defined until the organ has already acquired many of its most distinctive characters. The whole of the early nephridium is, however, strictly mesoblastic and the ectoderm plays no part whatever in its formation. Later in development, as will be shown, the ectoderm apparently contributes to the formation of the terminal vesicle; but as originally laid down in early development the entire organ is strictly mesoblastic.

The structure of the early nephridium, as present in the fourth

or fifth somite in advance of the growing zone, is shown in longitudinal section in Fig. 11, Plate 23. The anterior end of the organ extends across the thin ectodermal region just behind the segmental limit, and its lips are continuous with the remains of the primitive septum (not shown in Fig. 11). The organ consists of a straight unbranched tubule whose walls are composed of a continuous vacuolated protoplasm containing excretory granules. An axial ciliated lumen is present, which freely communicates with the body-cavity anterior to the septum by means of a minute opening through which the cilia project. Nuclei are present in the walls of the organ, which as yet shows no division into well-defined cells. As yet the lumen does not communicate with the exterior, although the posterior extremity of the organ is closely applied to the ectoderm and has apparently become continuous with the latter. Fig. 34, Plate 24 represents a tangential section through the ventro-lateral region of a larva of 13—14 somites, showing the two most anterior nephridia. The nephridia are the 2nd and 3rd larval nephridia (2nd provisional and 1st definitive, of somites V and VI respectively), the first pair of larval nephridia having already disappeared. The nephridia have increased in size considerably as compared with the stage represented in Fig. 11, Plate 23, but are otherwise essentially as described above. The posterior fusion with the body wall is here shown; the fusion takes place slightly behind and internally to the row of ventral hooked setae. The lumen is incomplete posteriorly. Anteriorly the continuity of the nephrostomial border with the septum is clearly shown.

At this period the nephridium is, in brief, a simple tubule with intracellular lumen, of the kind frequently described in other larval Annelids (cf. Meyer, 1887—88, Lang, 1889). It is peculiar, however, in being anteriorly in open communication with the body-cavity. In this peculiarity it presents a distinct contrast to the pronephridia described by Meyer and Lang, which are described as closed internally and as bearing a marked resemblance to the flame-cells of Turbellaria — a resemblance which has formed the basis of much phylogenetic speculation. The pronephridia of *Arenicola*, however, possess a distinct coelomic aperture of the kind described, as I have observed with perfect clearness an indefinite number of times. The anterior region of these organs in fact, together with a portion of the adjoining septum, constitutes the primitive nephrostome, from which the adult nephrostome is directly derived, as will shortly be shown.

It is noteworthy that at the stage represented in Fig. 34, Plate 24 the two foremost pair of nephridia are in all respects alike, although the anterior of the two later on completely degenerates and disappears, while the posterior continues its development and forms the first definitive nephridium of the adult. The first larval nephridium (in somite IV) has in most larvae already disappeared at this stage. The time of its disappearance is variable; in some larvae it has already disappeared at a stage of 9—10 somites, while in others it may persist in a recognizable form until fifteen or more somites have been attained. The second larval nephridium, which correspond to the first definitive nephridium of *A. marina*, also degenerates and disappears, but not until a considerably later stage.

(d) Later Development of the Nephridium.

The later development of the nephridium consists chiefly (1) in an increase in size and an accompanying division into well-defined cells with definite boundaries; (2) in a differentiation of the anterior region of the nephridium, and apparently also of a portion of the adjoining septum, to form the nephrostome, and in the establishment of characteristic relations with the blood-vessels; and (3) in the formation of the terminal vesicle. Each of these processes will be considered in order.

Subdivision into Cells.

In nephridia of the stage represented in Fig. 34, Plate 24 no definite cell-boundaries are visible, although the protoplasm has the typical vacuolated structure and contains excretory granules. It remains none the less continuous and undivided from end to end of the organ; its nuclei are small and as yet differ only slightly from the neighboring mesodermal and ectodermal nuclei of the body-wall. The lumen retains this intracellular character for a considerable period. Fig. 25, Plate 23 represents a cross section of the last nephridium (somite XI) of a more advanced larva than that of Fig. 34; the organ has increased considerably in size and its protoplasm, together with the large nucleolated nuclei, shows the structure characteristic of fully differentiated and functional excretory cells. As yet however, cell-limits have not appeared and the lumen remains intracellular. Fig. 37, Plate 24 represents a longitudinal section of the corresponding nephridium (somite XI) of a slightly more advanced larva in which the organ is beginning to show a subdivision into

cells. A cross section of a similar nephridium is represented in Fig. 26, Plate 23. The cells are large and few in number, and possess large nucleolated nuclei. At the narrower posterior region of many nephridia of this stage the lumen may remain intracellular, even although its anterior region may be intercellular and bounded by two or three distinct cells in cross section. As the cells increase in number, the entire lumen becomes intercellular. The distinction between the two forms of lumen seems thus purely artificial and a continuous gradation can be traced.

The subdivision into cells takes place apparently in simple relation to the increased calibre of the lumen of the organ. Accordingly it is found, as growth proceeds, that the lumen becomes bordered by a well-defined layer of cubical excretory cells whose number in cross section is in general directly proportional to the measure of the circumference of the lumen. Figs. 27 and 28, Plate 23 show successive stages in the formation of this epithelium. Fig. 28 represents a cross section through the body of a nephridium of almost the same stage as that of Fig. 42, Plate 25. Five cells are already visible in cross section, and their inner ends are beginning to project into the lumen in the manner characteristic of latter stages. Figs. 45, 46—54, Plate 25 and Fig. 6a, Plate 22 show still more advanced nephridia with a greatly increased number of cells in cross section.

Since the length of the nephridium in *Arenicola* is limited, never exceeding that of the somite to which it belongs, the only way in which it is possible to secure the necessary increase in the extent of the excretory surface is by an increase in the calibre of the organ. Since each nucleus is in direct physiological relation to only a limited portion of the excretory protoplasm, the increase in calibre entails a corresponding increase in the number of cells in cross section. The cells retain an approximately uniform size as may seen by comparing the above figures, and increase in number (apparently by direct division) as the epithelium increases in extent. Mitoses are never seen in the excretory epithelium; and it seems clear that the cells when they exceed a certain limit of size, undergo a simple subdivision, preceded by an amitotic division of the nucleus. I have not directly observed the several stages of this process: the nuclei, however, are frequently seen in an irregular or constricted form, and subdivision probably simply represents the extreme of such a condition. The formation of a cell wall between the two

nuclei thus formed would complete the process. The structure of the larval excretory cells varies but slightly at different stages. In the adult nephridium the cells have become very numerous and greatly compressed in a lateral direction, assuming a marked columnar structure; in consequence of this, apparently, the different regions of individual cells may present more marked differences from one another than are found in the broad cubical cells of the larval nephridium. In essential histological character, however, the larval and adult cells seem to differ but slightly from each other (see p. 370).

Nephrostome and its Associated Blood-Vessels.

In the formation of the nephrostome both the anterior region of the original nephridium and a portion of the adjoining septum take part. From its earliest appearance, as above shown, the nephridium possesses an anterior opening into the body-cavity of the next somite; but at first the marginal region bordering this opening is in no respect different from the remainder of the organ. When subdivision into cells begins the cells of the anterior region at first entirely resemble the others in structure and appearance; as growth proceeds, however, subdivision progresses more rapidly in these cells than in those behind and as a result the more anteriorly situated cells become smaller and more cubical. At the same time they lose their distinctively excretory character and the protoplasm becomes more homogeneous in appearance, while the vacuoles and excretory granules disappear (Plates 24 and 25, Figs. 37, 39, 42).

In Figs. 37 and 39, a portion of the adjoining septum appears to be assuming the character of a cubical epithelium and thus to be contributing to the extension of the dorsal lip of the funnel. The limits between septum and nephridium proper are in fact not sharply defined in early stages (Plate 24, Fig. 34); and until the funnel-epithelium is definitely formed, it is impossible to say where the two become continuous. Its hinder part however is unquestionably formed from the anterior cells of the nephridium proper (Figs. 37—39, 42), while its marginal cells, especially those of the dorsal lip, seem to be formed in the region of the original septum. The close relation of the dorsal lip to the septal structures, especially the blood-vessels (a relation which persists throughout life), is thus explained. The anterior portion of the nephridium becomes in this manner composed of an epithelium of small cubical ciliated cells (shown in

cross section in Plate 25, Figs. 43 and 44). The cilia of these cells become shorter and more numerous (compare Plate 24, Figs. 38, 39; Plate 25, Figs. 42, 46—49), while at the same time the nuclei become smaller and more rounded, and the nucleoli characteristic of the excretory cells usually disappear. Eventually a well-defined funnel region is formed whose cells are sharply distinct from the excretory cells behind (Figs. 46—49).

During the earlier stages of the above transformation the intersegmental septum undergoes changes which lead to the formation of the segmental blood-vessel. The septum at the beginning of the period in question extends obliquely forward from the ventral region of the stomach (where it is continuous with the sub-intestinal blood-vessel) to the ventral body-wall, forming a thin membrane which extends laterally to a point slightly above the level of the early nephrostome (Plate 25, Fig. 43). In its lateral portion its border becomes continuous with the lip of the early nephrostome, as above described — the anterior face of the septum being directly continuous with the epithelium of the funnel — while the posterior lamella passes directly into the peritoneum covering the nephridium. Figs. 37 — 39, Plate 24 show the relations of septum and nephridium at the early period of differentiation of the nephrostome before definite blood-vessels have appeared.

Each segmental blood-vessel appears in the septum (see Plate 25, Fig. 43, in which portions of the original septum yet remain), originally as a space between the two lamellae of the latter. The vessel begins its formation at the junction with the sub-intestinal blood-vessel (Plate 22, Fig. 9) and gradually extends outward to the body-wall where it joins the lateral longitudinal vessel, also formed about this time. (The relations of the segmental vessel to the nephridium are in part shown in Plate 25, Figs. 42—44). Near its junction with the body-wall the main vessel gives off a branch (the nephrostomial vessel) which curves back and passes inward and backward along the dorsal lip of the nephrostome between the funnel-epithelium and the peritoneum, to the glandular portion of the nephridium along which it passes as the main nephridial vessel. The nephrostomial vessel is thus directly continuous with the segmental vessel and is formed in essentially the same manner as this latter, representing in reality a space between the posterior septal lamella (represented by the peritoneum) and the anterior lamella at the region where the latter joins the funnel-epithelium with which it is

continuous. As the epithelium increases in extent the vessel acquires its definitive position just external to the margin of the dorsal lip.

In this way are defined the limits of the future dorsal and ventral lips, the former of which is distinguished by the presence of the blood-vessel which runs along its entire margin. At the inner future posterior angle of the nephrostome the vessel passes on to the body of the nephridium and gradually extends backwards to form the nephridial vessel. This vessel also represents a space between the peritoneum and the nephridial epithelium, and is formed as a simple continuation of the nephrostomial vessel. A slight peritoneal fold extending along the length of the nephridium (Plate 23, Figs. 25, 26) marks in early stages the position of the future vessel. In the more advanced nephridium represented in Fig. 27 the vessel has become well-defined in the anterior part of the nephridium, while posteriorly it is still represented by the peritoneal fold. Later the vessel completes its backward extension and forms, as well known, the chief vascular supply of the glandular region and terminal vesicle.

It is impossible in this place to give a full account of the manner in which all the blood-vessels of *Arenicola* arise, and the above brief account serves simply to explain the manner in which the characteristic relations arise between the segmental blood-vessel and the nephrostome. We have seen that the nephrostomial blood-vessel is formed as a portion of the system of septal blood-vessels: and that its association with the funnel depends simply upon the fact that this structure itself represents in large part simply a differentiated part of the septum[1].

Shortly after the appearance of the system of septal blood-vessels the membranous portion of the septum disappears[2]. The nephrostome then undergoes a change of position, its dorsal border assuming the antero-posterior direction of the nephridial blood-vessel, and the aperture consequently becoming directed inwards

[1] For the vascular supply of the adult nephridium see Gamble & Ashworth, 1900. Their Fig. 29 shows in a simple and clear manner the essential vascular relations of the nephridia. The detailed distribution of the blood-vessels of *A. cristata* has been the subject of investigation at the Marine Biological Laboratory, the results of which are not yet published.

[2] With the exception of a small portion extending from the anterior angle of the nephrostome to the body-wall and bearing the extra-nephridial part of the segmental vessel see Plate 22, Fig. 6a, cf. also Gamble & Ashworth 1900, Figs. 53—54).

Plate 25, Figs. 44, 46—49 as in the adult. The slip of transverse muscle which binds the nephrostome down to the body-wall has already appeared at the stage of Fig. 44, in a manner not satisfactorily understood. The slip however is associated with the segmental blood-vessel and is attached to the anterior border of the nephrostome near the point where the nephrostomial blood-vessel takes it rise. The fact of its attachment to the dorsal lip at the anterior angle of the funnel becomes thus to some degree intelligible.

The origin of the germ-cells, which arise in connection with the early nephridial vessels, may be briefly referred to here. Mere mention of the place and manner of origin of these cells will be sufficient, since the spermatogenesis and ovigenesis of *Arenicola* have been the subject of special investigation at Woods Hole by other authors an account of whose work will it is hoped appear before long.

The nephridial vessels in somites VII—X (2nd to 5th nephridia are associated with the formation of the gonads. The early germ-cells in connection with each nephridium become distinguishable soon after the appearance of the blood-vessel of the latter, and arise as a proliferation of the peritoneal cells of its wall. They appear first on the anterior and first-formed portion of the vessel, i. e. in the region immediately adjoining the posterior angle of the funnel, and at their first appearance (Plate 23, Fig. 27, have the form of somewhat enlarged vesicular nuclei surrounded by a small quantity of protoplasm and otherwise differing but slighty from the unaltered peritoneal cells. As growth proceeds the germ-cells increase in number and in size and acquire a very characteristic appearance Plate 25, Fig. 56). The nucleus becomes greatly enlarged and is surrounded by a well-defined protoplasm of homogeneous appearance Fig. 56 represents the appearance of these cells (oö-or spermatogonia) on the second nephridium of a larva of a well-advanced stage. The large vesicular nuclei, whose chromatin is arranged in scattered clumps closely applied to the nuclear membrane, already bear a close resemblance to the nuclei of the large oögonia or spermatogonia of the adult gonad. The germ-cells usually appear on their respective nephridia in the order of the formation of these organs, i. e. in order from before back. I have never found them in connection with the first or last pair of nephridia, which typically do not bear gonads in the adult.

The later development of the nephrostome consists essentially in the extension of its epithelial surface, and in the completion of the

characteristic relations of this epithelium to the blood-vessels. As explained above, the formation of the series of dorsal vascular processes, and also of the vesicle of the ventral lip, is in all probability primarily dependent upon the increase in epithelial surface. I have not however been able directly to study the early condition of these characteristic structures, which do not arise until a late stage of development and have not yet begun their appearance in the most advanced larvae at my disposal.

Terminal Vesicle.

The terminal vesicle — as might have been expected from the similarity of its adult structure to that of the glandular region — is formed as a differentiation of the most posterior portion of the primitive nephridium. There is no ectodermal invagination and from the resemblance in structure between the two regions it would appear a priori probable that the vesicle, like the tubular portion of the nephridium, is chiefly if not entirely of mesoblastic origin. A complete fusion, however, takes place at an early stage between the ectoderm and the posterior end of the nephridium, and it is from this region of fusion, in which the limits of ectoderm and mesoderm become indistinguishable, that the vesicle is differentiated. In all probability, therefore, its distal portion is derived from a region which originally was of ectoblastic origin; it seems however impossible to decide the extent to which each germ-layer has taken part in its formation. In point of fact the vesicle is differentiated as a whole, without regard to limits of germ-layers, from the extreme posterior region where the nephridium and the ectoderm become indistinguishably fused (see Plates 24 and 25, Figs. 34, 37—42).

Fig. 34, Plate 24 represents the early nephridia of somites V and VI in a larva of 14 somites. The nephridium is fused with the ectoderm slightly behind and internal to the ventral hooked setae at a point where the nephropore afterwards opens. At the point of fusion, ectoderm and mesoderm appear simply to become continuous with each other without any visible line of demarcation. The portion of the nephridium immediately adjoining the fusion remains similar to the rest of the organ until a relatively late stage (cf. Plates 24 and 25, Figs. 37—42). Fig. 40 represents a cross section through the posterior portion of the first nephridium of a larva of the full number of somites and a length of about 1,75 mm. The

wall of this region is vacolated and contains numerous coarse deeply-staining granules, which are also present in the adjoining ectoderm: but no sharply defined terminal vesicle is as yet present. In the nephridium represented in Fig. 42 2nd nephridium of a somewhat more advanced larva the conditions are essentially similar.

In Plate 25, Fig. 45, a representation of a cross section of a still more advanced stage, the earliest condition of the vesicle is seen. The terminal section of the nephridium has become dilated and has acquired thinner walls which however in all other respects are similar to those of the glandular regions proper. The strand of muscle-fibres which extends over the nephridium at the junction of the thick-walled glandular region represents a portion of the original longitudinal muscle-layer which has already become associated with the terminal region at the stage of Fig. 40. These muscle-fibres constitute the entire early musculature of the organ; later an extensive network of muscle-fibres is formed over the entire surface of the vesicle.

Figs. 52–55, Plate 25 represent a later condition of the vesicle, which has become much enlarged, and well defined with reference to the adjoining glandular region. The most striking characteristic of the vesicle in this larva was the presence of the large intensely staining granules, both in the wall of the vesicle and in the neighboring ectoderm (see Fig. 54). The presence of granules in the ectoderm of this region is a common phenomenon, and is somewhat difficult to understand. Possibly the granules in the preparation represent drops of fluid excretory matters which find their way between the ectodermal cells and collect there. No external opening at this stage can as a rule be detected, and the contractions of the vesicle may possibly force the excretory matters into the intercellular spaces of the ectoderm.

Fig. 6a, Plate 22 represents the vesicle at a later stage in a distended condition, showing the thin walls composed of flattened cells. In larvae of this stage the contractions of the vesicle may readily be observed through the transparent body-walls. In later development the number of epithelial cells increases greatly and the cells themselves become columnar (see above p. 370). The musculature of the wall also undergoes a great extension.

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Explanation of Plates 22—25.

All the figures of microscopic structure were drawn with the aid of the camera lucida under Zeiss objectives: Figs. 35 and 36 with 8 mm. obj., Oc. 6; Fig. 11 with 2 mm. apochromat., Oc. 1; Figs. 7, 8 and 29 with 2 mm. apochrom. Oc. 2; and the remainder with 2 mm. apochromat. Oc. 4. Finer details were usually drawn in with Oc. 6.

Reference Letters.

The number of the somite is indicated by a Roman numeral.

Each nephridium is designated by the letter n with an exponent to indicate its number in the larval stage, thus: n_2 = 2nd larval nephridium (nephridium of somite V) etc. The position of the septum is indicated by the numbers of the somites which it separates, thus: 5—6 = 5th septum.

b.w Body-wall.
c.c Chloragogen cells.
c.i.s Circum-intestinal blood-sinus.
c.m.f Circular muscle-fibres.
d_1, d_2 First and second diaphragms.
d.b.v Dorsal blood-vessel.
d.s Dorsal setae.
d.s.s Dorsal seta-sacs.
e.d.l Epithelium of dorsal lip of nephrostome.
e.g.z Ectoderm of growing zone.
e.v.l Epithelium of ventral lip of nephrostome.
ex.ep Excretory epithelium of nephridium.
ex.g Excretory granules.
g.c Germ cells.
gl.c Ectodermal gland-cells.
gl. neph Glandular region of nephridium.
g.z Growing zone.
int Intestine.
i.s Intersegmental septum.
l Leucocyte or excretophore.
l.b.v Lateral blood-vessel.
l.m.f Longitudinal muscle-fibres.
m.c Myoblast cells.
mn.c Mesenchyme cells.
m.p.n Mesoderm pole-nucleus.
m.s Muscular slip attached to dorsal lip of nephrostome.
mth Mouth.
m.t.v Muscle-fibres of terminal vesicle.
m.v.l Membranous wall of vesicle of ventral lip of nephrostome.
n.b.v Nephridial blood-vessel.
nst.ep Epithelium of nephrostome.
nst.v Nephrostomial blood-vessel.
oes Oesophagus.
pb Proboscis.
pd Proctodaeum.
p.d.l Ciliated processes of dorsal lip of nephrostome.
p.m Post-mesodermal region of posterior extremity.
pn Peritoneum.
pn.f Peritoneal fold forming nephridial blood-vessel.
prost Prostomium.
s Septum.
sb Seta-forming ectodermal cell ('setiblast').
s.b.v Segmental blood-vessel.
s.oe.g Supra-oesophageal ganglion.
st Stomach.
t.m.f Transverse muscle-fibres.
t.v Terminal vesicle.
v.b.v Ventral or sub-intestinal blood-vessel.
v.b.w Ventral body-wall.
v.s Ventral seta.
v.s.s Ventral seta-sac.
v.v.l Vesicle of ventral lip of nephrostome.

Plate 22. Arenicola cristata.

Fig. 1. Portions of three successive adult nephridia, showing general appearance after exposure by removal of transverse muscles. × 2.

Fig. 2. First adult nephridium of left side, with nephrostome turned upward and spread open so as to show structure of dorsal and ventral lips. × 7.

Fig. 3. Second adult nephridium of left side, seen in its natural position from above; showing nephrostome, with slip of transverse muscle attached to the anterior border of its dorsal lip; also position of nephrostomial blood-vessel and gonad. × 5.

Fig. 4. Fourth nephridium of left side with nephrostome freed from its attachment and turned outward so as to show the outer portion of the ventral lip. × 5.

Fig. 5. Portion of transverse section of wall of glandular portion of nephridium. Explanation in the text. × 800.

Fig. 6. Portion of transverse section of epithelial wall of the vesicular ventral lip of nephrostome, showing the abrupt transition to the membranous portion of the same. × 800.

Fig. 6a. Longitudinal section of first nephridium of a larva of 5—6 mm., cut tangentially to body-wall, showing terminal vesicle, membrane connecting nephrostome to body-wall, and nephridial blood-vessel; also structure of the excretory cells. Section passes below the nephrostome. × 800.

Fig. 7. Sagittal section through a larva of the swarming stage (somite V in process of formation), showing general characteristics of tissues at this stage. Nerve cord differentiated in ventral ectoderm; septa incomplete dorsally; early lumen of stomach indicated; mesoderm of the extreme posterior region in process of differentiation. × 800.

Fig. 8. Sagittal section through larva at beginning of crawling stage (somite VI in process of formation), showing early septa, growing zone, and early proctodaeum. Absorption of yolk has progressed considerably since stage of Fig. 7. × 800.

Fig. 9. Horizontal section through posterior region of larva in which somite VII is in process of formation, showing early differentiation of mesodermal structures, septa, ventral blood-vessel, chloragogen cells, nephridia. × 800.

Plate 23. Arenicola cristata.

Fig. 10. Oblique section through posterior region of larva in which somite IX is in process of formation, showing large posterior mesoblast nucleus, growing zone, early septa, early nephridial rudiments and ventral blood-vessel. × 800.

Fig. 11. Longitudinal section through body-wall of a larva in which somite VIII is in process of formation, showing early pronephridia of somites IV and 5. Goblet-cells in ectoderm also shown. × 800.

Fig. 12. Cross section through ventral portion of intersegmental region 2—3 of a larva in which somite VII is just appearing, showing large myoblast cells all similar to one another in appearance. × 800.

Fig. 13. Cross section through intersegmental region 5—6 of a larva in which somite VII is in process of formation, showing disposition and histological structure of mesoblast in region of differentiating 6th septum. Longitudinal muscle-fibres appearing. × 800.

Fig. 14. Transverse section through growing zone of a larva in which somite VI is in process of formation. Section passes somewhat obliquely forwards from above down. Dorsally the undifferentiated mesoblast is shown; ventrally the region of formation of early 5th septum and longitudinal muscle-fibres. Ectodermal cells large and well defined with large nuclei. × 800.

Fig. 15. Cross section through intersegmental region 5—6 in a larva in which somite VII is just appearing; showing on the right side the commencing differentiation of the third larval nephridium (somite VI); lumen just appearing in cells otherwise similar to the adjoining myoblast cells. Ectoderm cells well defined. On left side is shown early condition of dorsal and ventral seta-sacs. × 800.

Fig. 16. Cross section through anterior of somite IV of a larva in which somite IX is just appearing. 1st provisional nephridium shown in cross section. Myoblast cells reduced. × 800.

Fig. 17. Similar cross section through somite V of the same larva. × 800.

Fig. 18. Similar cross section through somite VI of same larva. × 800.

Fig. 19. Similar cross section through somite VII of same larva. On left side nephridium further advanced than on right. Muscle-fibres less developed than in preceding somites, and myoblast cells larger. × 800.

Fig. 20. Similar cross-section through somite VIII of the same larva. On the left the section passes more anteriorly than on right and there shows intersegmental region 7—8 with myoblasts. Nephridal cell not yet distinguishable from the others. Shows also early seta-sacs which still retain connection with the ectoderm and to which flattened mesoderm cells are applied. Muscle-fibres less developed than in preceding somite. × 800.

Fig. 21. Section through region of formation of somite IX of the same larva. Ventrally the section passes through region of formation of 8th septum; dorsally through the anterior border of the pigmented post-mesodermal region. Large well-defined cells of the ectodermal growing zone. Longitudinal muscle-fibres just making their appearance. × 800.

Fig. 22. Cross section through post-mesodermal region of same larva, showing small, pigmented, vacuolated, radially arranged ectoderm cells with their small deeply staining nuclei. A few flattened mesoderm cells applied to body-wall and proctodaeum. Strong cilia on ventral wall of proctodaeum. × 800.

Fig. 23. Cross section through anterior region of somite XI of a larva in which somite XII is in process of formation; showing commencing differentiation of 8th larval nephridium 6th definitive. Nephridium somewhat more advanced in development on the left side. Myoblast cells well defined but apparently undergoing reduction. Large embryonic nuclei in ectoderm; smaller nuclei in the ventral region have the characters of tissue nuclei, and are probably connected with the differentiating nerve-cord. × 800.

Fig. 24. Cross section through intersegmental region 11—12 of a larva in which somite XII is appearing; showing early mesoblast; intestine surrounded by blood-sinus continous with dorsal and ventral blood-vessels; large well-defined ectoderm cells of the growing zone. Differentiation of the ventral nerve-cord is in progress, and smaller, more deeply staining nuclei are seen in its vicinity. × 800.

Fig. 25. Cross section through nephridium and adjacent body-wall of somite XI of a larva of ca. 1.5 mm. length and ca. 45 somites. Nephridium (6th definitive of right side) still apparently one cell in cross section; showing also a peritoneal fold running along nephridium from septum in a position corresponding to the later nephridial blood-vessel. Nucleus large and nucleolated. Protoplasm vacuolated and containing granules; stains more deeply at border of lumen. Cross-cut cilia visible in lumen. × 800.

Fig. 26. Cross section through nephridium and adjacent body-wall of somite VIII of a larva of a later stage than that of Fig. 25 (ca. 1.6 mm.; ca. 50 somites). Nephridium (3rd definitive of left side) has become divided into cells and lumen has become intercellular; 3 cells present in cross section at this region. Histological characteristics of cells same as in Fig. 25. A similar membrane occupies position of future nephridial blood-vessel.

Fig. 27. Cross section through nephridium and adjacent body-wall of somite VIII (3rd definitive nephridium, left side) of a more advanced larva than that of Fig. 26 (ca. 2,0 mm.; full number somites); section passes just behind nephrostome and shows nephridial blood-vessel with an early germ cell applied to it. × 800.

Fig. 28. Similar cross section through middle region of 1st definitive nephridium (somite VI) of right side of a larva of later stage than that of Fig. 27 (2.5 mm.; full number somites). Nephridium 5 cells in cross section; histological structure as above. Nephridial blood-vessel present in anterior portion of nephridium. × 800.

Fig. 29. Horizontal section through posterior body-region of a larva in which somite XI is being formed. Section passes along level of nephridia and ventral setae, and shows the entire series of early nephridia. × 400.

Fig. 30. Sagittal section through posterior body-region of a larva in which somite XI is being formed. Shows early septa, intestine, nerve-cord. Septa in advance of somite VIII are dorsally incomplete. Shows also large terminal mesoblast-nucleus. × 800.

Fig. 31. Longitudinal section through early region of formation of nephridia in a larva in which somite XI is just appearing; showing early septa and associated nephridial rudiments of the 6th and 7th larval nephridia (somites IX and X). Pole-nucleus of mesoderm and mitosis in undifferentiated mesoderm cells also seen. Also the large ectodermal nuclei of the growing zone. × 800.

Plate 24. Arenicola cristata.

Fig. 32. Nearly horizontal section through the posterior region of a larva in which somite XVI is in process of formation. The ectodermal growing

zone, and the differentiation of the seta-sacs and early septa are shown. The proctodaeal cilia in the post-mesodermal region are shown; also the mesodermal nuclei applied to the proctodaeum and body-wall of this region. × 800.

Fig. 33. Horizontal section through the posterior achaetous region of a more advanced larva (ca. 1.4 mm. length; ca. 45 somites) showing the growing zone, and formation of early septa of this region. Post-mesodermal region sharply defined; a valve separating proctodaeum and rectum; circular muscle-fibres applied to body-wall and anal region in this region. × 800.

Fig. 34. Part of a horizontal section of a larva in which somite XIV is appearing. Section passes tangentially to median ventral region a little to left of nerve-cord. Shows early nephridia of somites V and VI (2nd provisional and 1st definitive nephridia), and their relations to septa, ventral hooked setae and body-wall. × 800.

Fig. 35. Sagittal section of a larva in which somite VIII is forming; showing early septa, incomplete dorsally. First septum distinctly separate from retractor muscle of proboscis. Diaphragms have not yet appeared. × 300.

Fig. 36. Sagittal section of a larva in which somite XIV is forming, showing the series of septa and early stage of 2nd diaphragm. Remains of 3rd primitive septum seen stretching from insertion of diaphragm to anterior region of stomach. × 300.

Fig. 37. Horizontal section of left nephridium of somite VI (1st definitive in larva of ca. 2 mm. and 50 somites, showing early condition of nephrostome, and its continuity with the septum. × 800.

Fig. 38. Portion of a horizontal section through a more advanced larva than the preceding 1.5 mm. long; 50 + somites; showing nephridium of somite IX (fourth definitive), nerve-cord, and body-wall, cut tangentially. Posteriorly the nephridium is fused with the ectoderm; no terminal vesicle is as yet differentiated. Continuity of the nephrostomial lip with the primitive septum, and connection of the latter with the ventral blood-vessel, are visible. The smaller cells at the anterior end of the nephridium form the early nephrostomial epithelium. × 800.

Fig. 39. Sagittal section through nephridium and adjoining body-wall of somite VI (1st definitive nephridium) of a larva of about same stage as last (ca. 45 somites; ca. 1.7 mm. long). Showing continuity of septum with dorsal lip of early nephrostome. The most anterior cells of the nephridium are small and cubical; closely crowded nuclei in the portion of the septum immediately continuous with the dorsal lip probably indicate a stage in the transformation of this portion of the septum into funnel-cells. No differentiated terminal vesicle as yet. Nephridial blood-vessel not yet formed. × 800.

Fig. 40. Cross section through posterior extremity of 1st nephridium (somite VI) of a larva of a more advanced stage than that of Fig. 38 (ca. 2.25 mm.; full number of somites), showing the posterior continuity of nephridium and ectoderm and the presence of excretory granules in the latter. No differentiated terminal vesicle as yet. The terminal region is overarched by a band of muscle-fibres continuous with the longitudinal

fibres of the body-wall. These represent the first appearance of the musculature of the terminal vesicle (compare Figs. 45, 53, 55). × 800.

Fig. 41. Horizontal section through ventral region of somites VII and VIII of a more advanced larva (ca. 3 mm.; full number of somites), showing the origin of the segmental vessels from the ventral blood-vessel, and the relations of one of the vessels to the 2nd nephridium of the right side. × 200.

Plate 25. Arenicola cristata.

Fig. 42. Longitudinal section through the left nephridium of somite VII (2nd definitive nephridium) and adjacent body-wall of the larva of Fig. 41, showing (1) a portion of the nephridial blood-vessel to which is applied a large germ-nucleus; (2) the posterior junction of the nephridium with the ectoderm corresponding to the early undifferentiated terminal vesicle); and (3) the cubical cells of the early nephrostome with their shorter cilia. The lumen has extended throughout the entire length of the organ and seems to communicate with the exterior; the external opening, however, is very minute. × 800.

Fig. 43. Cross section through the anterior end of the first left definitive nephridium (somite VI) of a larva of about the same stage as Fig. 42, showing the early condition of the septal blood-vessels of this somite, and their relations to the nephridia and to the body-wall. Portions of the primitive septum still remain. × 800.

Fig. 44. Longitudinal section through the anterior part of the 4th nephridium (somite IX) of a larva of a somewhat more advanced stage than Fig. 43 (length ca. 3 mm.). The nephrostome is turned somewhat inward and has been cut in such a way as to show its epithelium in cross section; the figure shows also its relations to the nephrostomial blood-vessel, which runs across its dorsal lip and is in close relation to the transverse band of muscle (*t. m. f.*), which is also attached to this lip and binds it down to the ventral body-wall. A portion of the primitive septum in which the blood-vessel appears is seen at *s.* × 800.

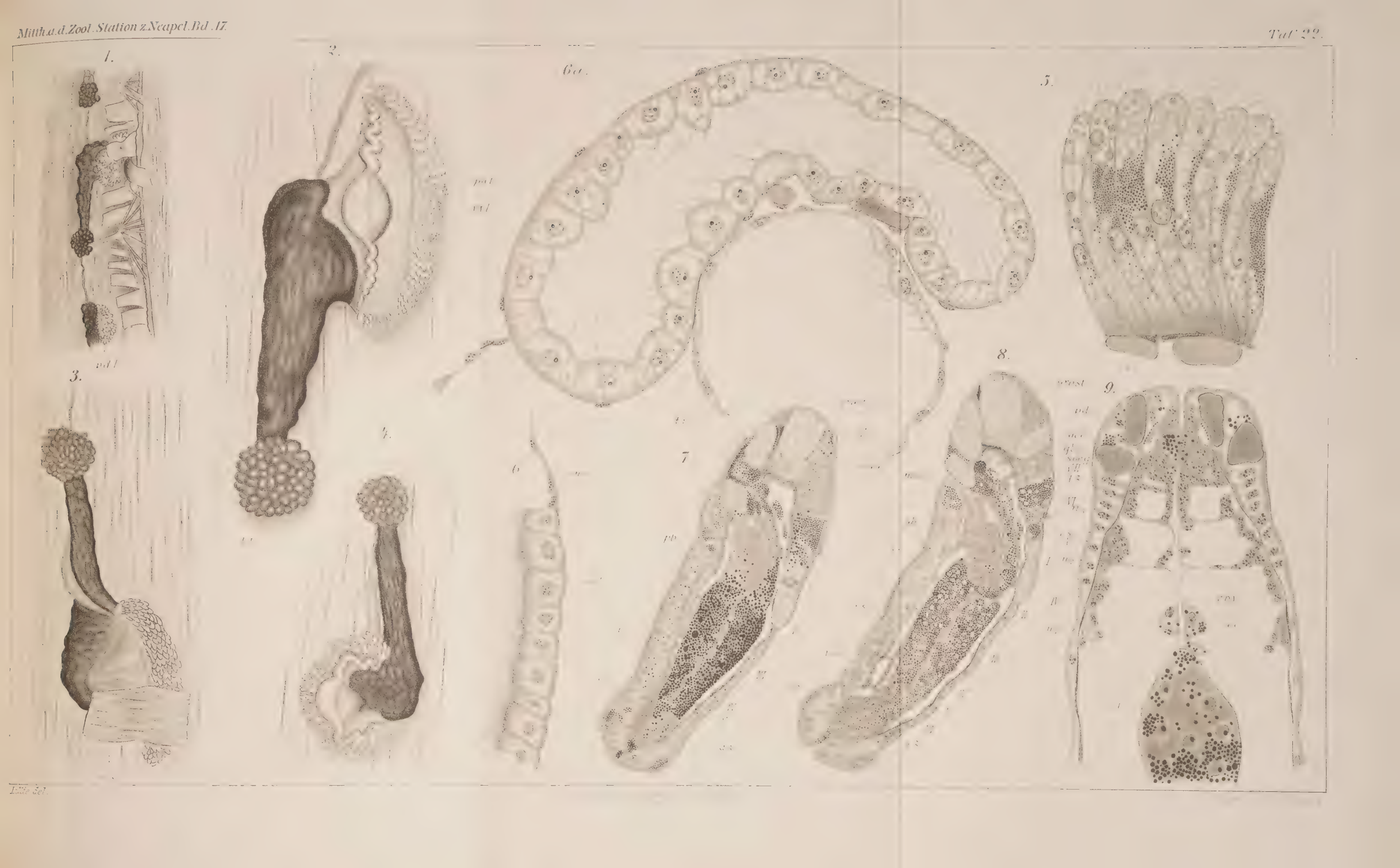
Fig. 45. Cross section through the posterior end of the 1st nephridium (somite VI) of a larva in which the terminal vesicle is beginning to differentiate (length ca. 3.5 mm; full no. of somites). The terminal portion adjoining the ectoderm is thin walled and somewhat dilated. A band of muscle-fibre (derived from the longitudinal layer of the body-wall) extends across the terminal vesicle at its junction with the glandular region proper. Granules in the ectoderm near the terminal vesicle. × 800.

Figs. 46-50. These figures represent successive transverse sections, 7.5 μ thick, through the nephrostome and anterior glandular portion of the first nephridium (somite VI) of a larva of ca. 4 mm. in length. The nephrostome has become well-defined and is composed of a single layer of small cubical ciliated cells; along its dorsal lip runs the nephrostomial blood-vessel. The continuity of this vessel with the nephridial vessel is shown. The relation of the segmental blood-vessel to the transverse muscle-band attached to the dorsal lip is also shown. *l*, vacuolated wandering cells (excretophores?). × 800.

Figs. 51-54. These figures similarly represent four successive sections of the terminal vesicle of the same nephridium. Large deeply staining granules are present in the walls of the vesicle, and in the adjacent ectoderm of the body-wall. × 800.

Fig. 55. Cross section of the terminal vesicle and adjacent glandular region of the 2nd nephridium of the left side somite VII of the same larva. The flattened cells of the vesicle with their contained excretory granules are shown. Also the early muscle-band crossing the vesicle at its junction with the glandular region. × 800.

Fig. 56. Cross section through the anterior region of the nephridium of Fig. 55, immediately behind the funnel, showing the nephridial blood-vessel with well-defined germ-cells (rudimentary ovary or testis) applied to it. × 800.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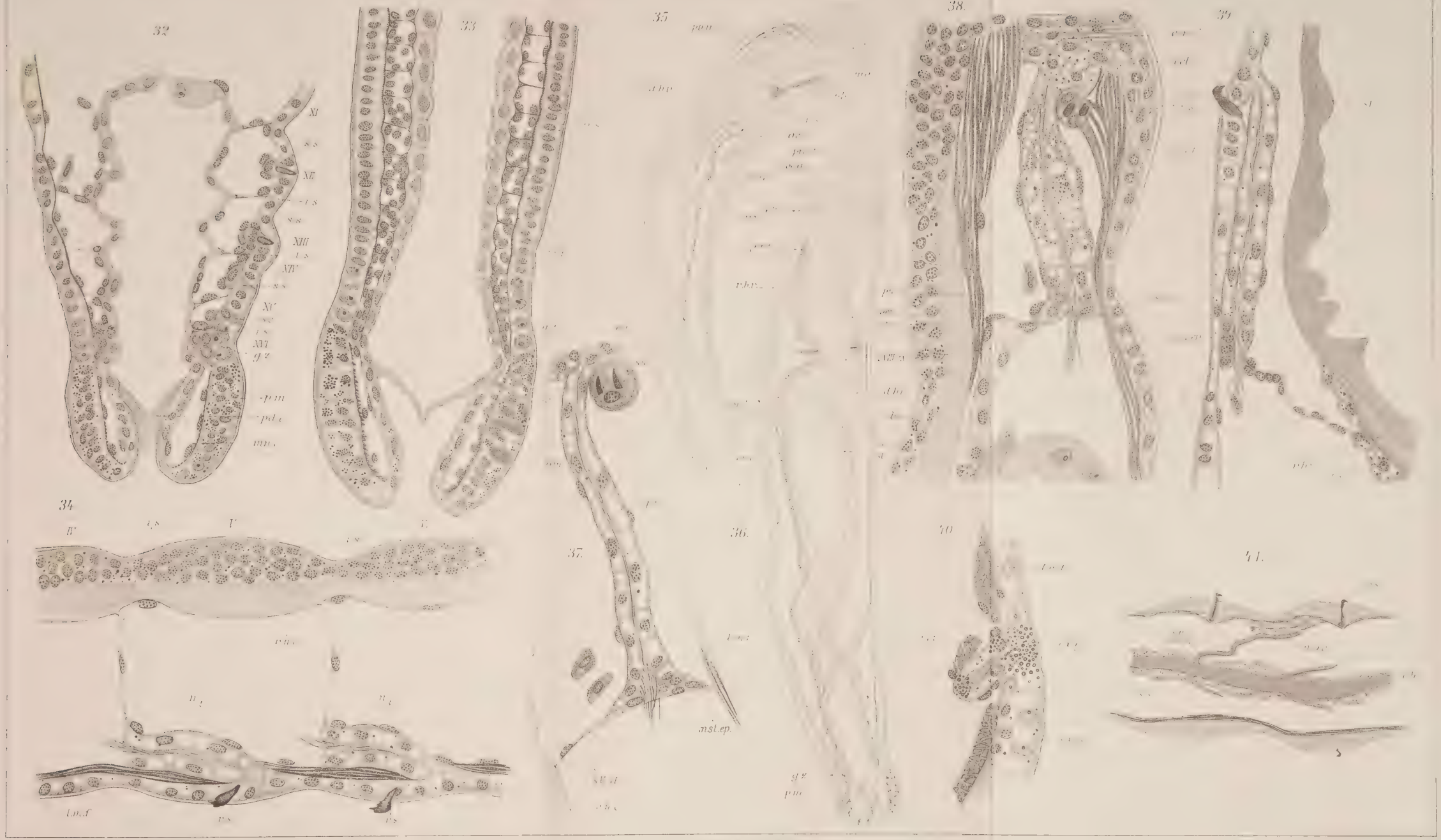
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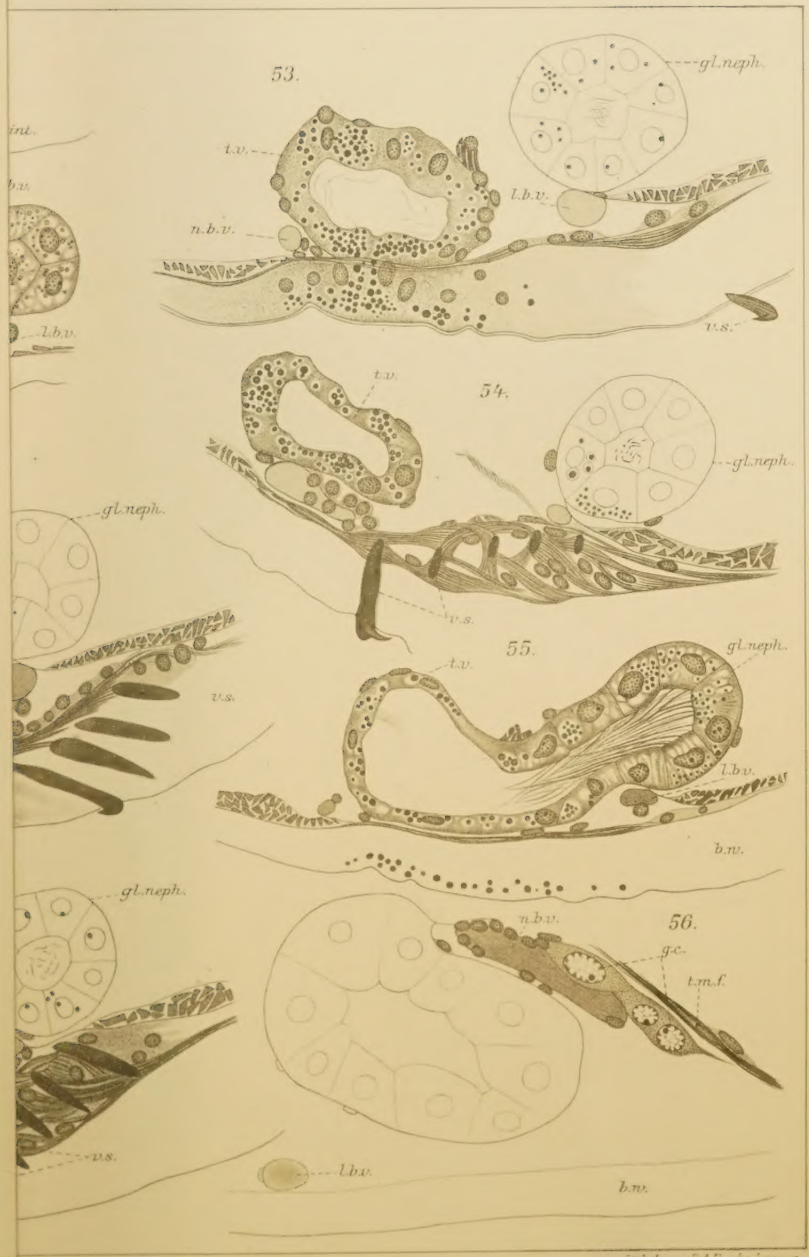

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